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Award Number: DAMD17-01-1-0350

TITLE: An Investigation of Antioxidant Supplements and Medicinal Herbs in Breast Cancer Recurrence and Survival

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REPORT DATE: September 2002

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2002	Annual (1 Sep 01 - 31 Aug 02)

4. TITLE AND SUBTITLE
An Investigation of Antioxidant Supplements and Medicinal Herbs in Breast Cancer Recurrence and Survival

5. FUNDING NUMBERS
DAMD17-01-1-0350

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***
There is urgent need to resolve the question of whether high consumption of antioxidants from diet and/or supplements can impact the prognosis of women diagnosed with breast cancer. A meta-analysis was conducted to summarize previous findings for high intakes of vitamin C from diet and supplements and risk of breast cancer-related mortality. Relative risk estimates for highest vitamin C consumption and breast cancer survival ranged from 0.40 to 0.92. The random effects estimate for the highest intake of vitamin C and mortality was 0.72 (95% CI, 0.57-0.90). In addition, proportional hazards and logistic regression modeling were used to estimate rate ratios and odds ratios for recurrence and/or mortality among 385 postmenopausal women diagnosed with breast cancer between 1986 and 1988 enrolled into a case-control study on diet and cancer. One or more supplements were used by 80.5% of women, and use increased significantly after diagnosis of breast cancer. Antioxidant supplement users compared with non-users were less likely to have a breast cancer recurrence or breast cancer related death (OR=0.54; 95% CI, 0.27-1.04). Vitamin E supplements showed a modest protective effect on recurrence and mortality when used for more than 3 years (OR=0.33; 95% CI, 0.10-1.07). Biases may have influenced these results. This study provides limited support for the hypothesis that antioxidant supplements may reduce the risk of breast cancer recurrence or breast cancer related mortality.

14. SUBJECT TERMS:	15. NUMBER OF PAGES
breast cancer, antioxidants, post-menopausal, survival	66
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Re: Annual Summary Report for Award Number DAMD17-01-1-0350

Introduction:

Antioxidants have been shown to reduce the risk of cancer in laboratory and epidemiological studies, possibly through their ability to neutralize free radical damage (1). In breast cancer, vitamin C and E supplements may also induce mammary cell differentiation (2), apoptosis (3, 4), and may inhibit tumor progression (5). Several epidemiological studies have shown inverse associations for vitamins C and E from diet and/ or supplements and breast cancer (6-9), whereas others have reported no relationship (10,11). Few studies however, have examined the associations between antioxidants and post-diagnosis outcomes. Dietary intakes of vitamins C and E from foods were protective in three case-control studies of breast cancer mortality (12-14), but they were found not to be related to risk in a single case-control (15) and in one large cohort study (16).

This DoD sponsored project was an epidemiological investigation into the associations among antioxidant supplementation in women with breast cancer and risk for breast cancer recurrence and cancer-related death. The study served as a follow-up to the Fatty- Acids, Tumor Characteristics and Carcinoma of the Breast (FASTCAB) study. FASTCAB is a comprehensive, interdisciplinary case-control study examining nutritional biomarkers, histopathologic tumor characteristics and genotypes in 407 Boston-area postmenopausal women diagnosed with breast cancer. The follow-up study questionnaire, administered at least 12 to 14 years since diagnosis for each case, enabled comparisons of supplement usage before and after diagnosis of breast cancer, and associations with breast cancer recurrence and survival. The project provided for assessment of relative risk (hazard ratios) of antioxidants for breast cancer recurrence and cancer-related and overall mortality.

Our central study hypothesis, based on prior epidemiological research indicating a protective role for vitamin C intake and breast cancer survival, was that antioxidants supplements might be related to prevention of breast cancer recurrence and disease-related mortality. In summary, this study used a well-defined study population, comprised particularly of understudied, older cancer patients to examine the effectiveness of antioxidant supplements in breast cancer recurrence and survival. While a dearth of evidence exists on the question of post-diagnosis supplementation, it is of urgent need to examine whether breast cancer patients should be using antioxidant supplements, particularly during the crucial time periods of cancer treatment. This study lends some evidence to this important question.

Training and Research Accomplishments (*Associated with Statement of Work*):

I. Designed, pilot tested and mailed study questionnaire.
   a. *Deviation in Statement of work: The Coloraine, Ireland component of this research plan was not performed due to International approval difficulties: these cases were not included in this study.*
   b. Questionnaire mailed to Boston Cohort only

II. Repeated mailing (two repeat mailings), phone-contacted recipients who had not mailed in questionnaire, then collected, coded and entered data from questionnaires into created database (SAS).

III. Analyzed data using several methodologies.
   a. Descriptive epidemiology
   b. Advanced multivariate models
      i. Logistic regression
      ii. Cox proportional hazards model

IV. Three manuscripts completed.
   a. A meta-analysis and critical review of vitamins C & E intake and the risk of breast cancer related mortality among survivors.
   b. Change in antioxidant supplement use after diagnosis and factors associated with use among early-stage postmenopausal breast cancer patients.
   c. Antioxidant supplements and risk of breast cancer recurrence and breast cancer related mortality among postmenopausal women.
   d. *Deviation in Statement of work: The focus for this project involved into a more in-depth analysis of antioxidants, particularly vitamins C and E. While limited data was presented for medicinal herbs, due to low frequencies of use, multivariate models for associations with outcome variables and use of medicinal herbs was not performed. Instead we present only descriptive characteristics for medicinal herb use in this population.*

V. Dissertation defense and approval of final dissertation.

VI. Submission of approved dissertation to the Graduate School.

VII. Presentation at conferences:
   a. AACR, 2002: American Association for Cancer Research, San Francisco, CA.
   b. Era of Hope, 2002: Department of Defense Breast Cancer Research Program, Orlando, FL.

List of Key Accomplishments:

- Fleischauer AT, Simonsen N, Arab L. Antioxidant supplements and risk of breast cancer recurrence and breast cancer related mortality among postmenopausal women. *Nutr Cancer* (Submitted).
- Fleischauer AT, Simonsen N, Arab L. (Abstract) Antioxidants and breast cancer survival. *Proceedings of the American Association for Cancer Research*, 2002.
- PhD defended March, 2002
- Degree awarded May, 2002
- Post-graduate Fellowship: Epidemic Intelligence Service, Centers for Disease Control and Prevention, June 2002 – Present.

Reportable Outcomes: Abstracts (*see Appendix 1 for complete manuscripts*)**:**

1. Title: A meta-analysis and critical review of vitamins C & E intake and the risk of breast cancer related mortality among survivors.

Background: There is urgent need to resolve the question of whether high consumption of antioxidants from diet and/or supplements can impact the prognosis of women diagnosed with breast cancer.
Design: A critical review of the literature on vitamin C, E and breast cancer recurrence and survival was performed. In addition, a meta-analysis was conducted to summarize previous findings for high intakes of vitamin C from diet and supplements and risk of breast cancer-related mortality.
Results: Eleven clinical or epidemiological studies examined the association between antioxidants consumed either before or after a diagnosis of breast cancer and subsequent risk of recurrence or mortality. Of these, seven were prospective cohort studies that examined vitamin C consumption with relation to breast cancer survival and reported relative risk (RR) estimates for the highest level of consumption. Relative risk estimates for highest vitamin C consumption and breast cancer survival ranged from 0.40 to 0.92, with median values for upper categories of intake ranging from 100 mg/day to 280 mg/day. The random effects estimate for the highest intake of vitamin C and mortality was 0.72 (95% CI, 0.57-0.90), with a p-value for heterogeneity of 0.38, and for a 100mg/day increase in vitamin C intake the effect was 0.65 (95% CI, 0.40-1.07).
Discussion: A meta-analysis across seven prospective studies suggests a protective association between high, predominantly pre-morbid vitamin C consumption from diet and risk of breast cancer mortality. Although the few studies that have to date reported RR estimates for antioxidants and breast cancer prognosis have been relatively consistent, possible alternative hypotheses that might explain the effect such as confounding by total fruit and vegetable consumption may preclude sole reliance on summary effect estimates.

2. Title: Change in antioxidant supplement use after diagnosis and factors associated with use among early-stage postmenopausal breast cancer patients.

Background: Dietary supplement and medicinal herb use is more prevalent among breast cancer patients than among the general US population. However, few studies have quantified the change after diagnosis and identified the characteristics of supplement use among postmenopausal breast cancer patients.
Methods: We re-contacted 385 women diagnosed with breast cancer between 1986 and 1988 who were enrolled in a case-control study on diet and cancer with a questionnaire to ascertain use of nutritional supplements and medicinal herb products before and during 12 to 14 years of post-diagnosis follow-up time. Multivariate logistic regression was performed to ascertain factors associated with use of supplements.
Results: One or more supplements or medicinal herbs were used by 80.5% of women. Use of supplements increased significantly after diagnosis. Antioxidant supplement use (vitamin C, E, beta-carotene, selenium or an antioxidant combination) increased from 34% pre-diagnosis to 56% after diagnosis, and was overall used by 64% of this cohort at some time during their adult life. Current exercise (OR=2.62, 95% CI=1.2-5.9) and concurrent use of medicinal herbs were

positively associated with new use of antioxidant supplements after diagnosis. Whereas increasing age was associated with supplement use prior to diagnosis, and opposite direction was observed post-diagnosis.
Discussion: Antioxidant, vitamin and mineral, and medicinal herb supplement use were highly prevalent among women with breast cancer, more so than in the general population. The increase in use of antioxidant supplements and medicinal herbs after diagnosis of breast cancer is a public health concern because of adverse effects and uncertain efficacy.

3. Title: Antioxidant supplements and risk of breast cancer recurrence and breast cancer related mortality among postmenopausal women.

Background: Despite widespread use, only a few clinical or epidemiological studies have examined the relationship between antioxidant supplements and risk of breast cancer recurrence or breast cancer-related mortality.
Methods: Proportional hazards and logistic regression modeling were used to estimate rate ratios and odds ratios for recurrence and/or mortality among 385 postmenopausal women diagnosed with breast cancer between 1986 and 1988 enrolled into a case-control study on diet and cancer. Women with previous participation were re-contacted with a questionnaire to ascertain the use of nutritional supplements during 12 to 14 years of post-diagnosis follow-up time.
Results: Antioxidant supplement users compared with non-users were less likely to have a breast cancer recurrence or breast cancer related death (OR=0.54; 95% CI, 0.27-1.04). Exclusion of proxy questionnaires for women who had died during follow-up (N=47) resulted in a slight lessening of this effect 0.60 (95% CI, 0.16-2.46). Vitamin E supplements showed a modest protective effect on recurrence and mortality when used for more than 3 years (OR=0.33; 95% CI, 0.10-1.07), with again a lessening of effect after removal of proxy respondents (RR=0.52; 95% CI, 0.15-1.83). Pre-morbid dietary intake of vitamins C or E from diet and/or supplements showed no relationship with risk. Multivitamins and duration of multivitamin use were also not associated with risk.
Conclusion: Risks of recurrence and disease related mortality were reduced among women using antioxidant supplements and vitamin E supplements for more than 3 years. Recall bias among proxy respondents may have partly contributed to these findings. This study provides limited support for the hypothesis that antioxidant supplements may reduce the risk of breast cancer recurrence or breast cancer related mortality.

Conclusions

The findings from this study lend limited evidence for the hypothesis that antioxidant supplements reduce the risk of breast cancer recurrence and breast cancer related mortality. Pre-morbid dietary intake of antioxidant supplements did not appear to be related to subsequent risk of recurrence or death, nor were pre-morbid vitamin C and E from both diet and supplements combined. Increasing lengths of use of vitamins C or E supplements appeared to be associated with a modest reduction in risk, but it is unclear what influence bias and error may have played with these finding. Nonetheless, vitamins C and E from diet or supplements have not shown a detrimental effect on breast cancer prognosis. Future studies should examine more closely supplementation based on frequency and dose as opposed to use versus non-use. And, more

research is needed to establish whether use of antioxidant supplements after diagnosis confers clear-cut prognostic benefits for women with diagnosed breast cancer.

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Appendix

Manuscript drafted:

1. A meta-analysis and critical review of vitamins C & E intake and the risk of breast cancer related mortality among survivors.

2. Change in antioxidant supplement use after diagnosis and factors associated with use among early-stage postmenopausal breast cancer patients.

3. Antioxidant supplements and risk of breast cancer recurrence and breast cancer related mortality among postmenopausal women.

A meta-analysis and critical review of vitamins C & E intake and the risk of breast cancer related mortality among survivors.

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Key words: Antioxidants, Breast Cancer Survival, Breast Cancer Recurrence.
Running Title: Vitamins C and E and Breast Cancer Survival

Abstract

There is urgent need to resolve the question of whether high consumption of antioxidants from diet and/or supplements can impact the prognosis of women diagnosed with breast cancer. A critical review of the literature on vitamin C, E and breast cancer recurrence and survival was performed. In addition, a meta-analysis was conducted for high intakes of vitamin C from diet and supplements. Eleven clinical or epidemiological studies examined the association between antioxidants consumed either before or after a diagnosis of breast cancer and subsequent risk of recurrence or mortality. Of these, seven were prospective cohort studies that examined vitamin C consumption with relation to survival. Relative risk estimates for highest vitamin C consumption and breast cancer survival ranged from 0.40 to 0.92, with median values for upper categories of intake ranging from 100 mg/day to 280 mg/day. The random effects estimate was 0.72 (95% CI, 0.57-0.90), with a p-value for heterogeneity of 0.38. A meta-analysis across seven prospective studies suggests a protective association between high dietary vitamin C consumption and risk of breast cancer mortality. Although the few studies that have to date reported RR estimates for antioxidants and breast cancer prognosis have been relatively consistent, possible alternative hypotheses may preclude sole reliance on summary effect estimates.

Introduction

With increasing breast cancer survival rates [1], women diagnosed with the disease have begun to modify their diets in favor of higher intakes of fruits, vegetables and antioxidant supplements in order to potentially alter their breast cancer prognosis [2]. This underlines the need to resolve the question of whether high consumption of antioxidants from diet and/ or supplements can affect the prognosis of women diagnosed with breast cancer. To date, there exists conflicting evidence that antioxidants from diet, supplements or from measurements in blood are related to incident breast cancer, breast cancer recurrence or breast cancer related mortality [3].

Among the dietary antioxidants, vitamin C has received decades of interest and has been postulated to decrease the risk of cancer in general [4], and breast cancer in particular [5,6]. Case-control studies on vitamin C and breast cancer have supported this relationship [7], while large cohort studies failed to identify significant benefit [8-10].

Motivated by previous epidemiologic studies of vitamin C and incident breast cancer as well as clinical and laboratory studies, we report results from a meta-analysis of the relationship between pre-diagnosis vitamin C intake from diet and subsequent risk of breast cancer-related mortality. This review examines the epidemiology on antioxidants from diet and supplements with regards to post-diagnosis outcomes, such as recurrence, treatment failure and breast cancer-related mortality.

Methods

Epidemiological and clinical studies included in this review were identified through repeated MEDLINE literature searches conducted up to January 2002. The reference terms, "antioxidants", "supplements", "vitamins", "diet", "nutrition" with "breast cancer recurrence", "disease-free survival" and "breast cancer survival", were used as both keyword and subject terms. The search was limited to human studies published in English. In addition, journal articles cited in the primary-search manuscripts were collected and added to the review.

Seven cohort studies were found which reported relative risk (RR) estimates for vitamin C intake and risk of breast cancer-related mortality. These studies included North American, Swedish and Australian cohorts. The four remaining studies, which included the two clinical trials [22,23], a descriptive study [24] and a cohort study of vitamin C and treatment failure [25] were not included in the meta-analysis. The clinical trials were not included because they were not randomized and vitamin C was used in conjunction with other supplements. The descriptive study was not included since it was an ecological study design and not on the level of individual data. The study of Holm et al [25] was also not included since the outcome for the meta-analysis was survival. The adjusted relative risk estimate (RR), confidence interval (CI), and/ or p-value, year of publication, number of subjects, antioxidant consumption levels, and covariates controlled for in analyses were abstracted from these references. From each published report, RR estimates and 95% CIs for the highest category of vitamin C intake compared with the lowest were extracted.

A meta-analysis of dietary consumption of vitamin C and breast cancer survival of the published epidemiologic studies was performed. The standard errors (SE) were calculated from the extracted RR estimates and 95% CIs by using the following equation: $SE= [(ln\ RR_{upper}) - (ln\ RR_{lower})]/3.92$ [26]. However, when the 95% CI was not reported, an estimate of the SE was calculated from an exact *P* value by using the following equation: $SE=[ln\ RR]/ z$, where *z* is the *z*

score of the normal distribution for the exact *P* value [26]. Average differences in vitamin C consumption between the highest category of intake and the reference group were computed by assigning a midrange dose to each category and averaging values across all reports. Fixed- and random-effect estimates were calculated by using inverse variance weighting [27], and assuming that the effect measures in the study populations have a common mean value. Random effect estimates were used because they add an additional component of among-study variance to each study-specific estimate, which allows for two sources of variability, within and between studies [27]. Tests for homogeneity were conducted for each analysis. Details of the studies are presented in Tables 1and 2 by outcome and study design. Due to the limited number of studies, a meta-regression using the aforementioned extracted variables was not performed.

Results

For the critical review of the literature, the following studies, based on the time period in which antioxidants were assessed, were divided into either pre-morbid or post-diagnosis and by outcome (survival or recurrence) for the epidemiologic cohort studies. Descriptive and intervention studies are discussed in latter sections. We found 11 studies that examined antioxidants and breast cancer prognosis. Aside from the protective effect highlighted in the following meta-analysis for vitamin C and breast cancer survival, the evidence regarding other antioxidants, vitamin E or β-carotene is too limited to conduct a meaningful quantitative assessment. Therefore, reliable conclusions cannot be drawn.

In order to examine whether the associations between differed according to the cut-points used for the highest levels of vitamin C intake, figure 1 plotted RR estimates for the highest category of consumption compared with the study-specific reference level. Hebert et al. [28] reported the RR as per 100 mg/day of vitamin C. Saxe et al. [29] reported a median value of vitamin C intake among cases of 161 mg/day. Ingram et al. [30] and Holmes et al. [31] did not report quantitative cut-points for 'high' consumption or for the highest quantile, respectively. The remaining values were computed by calculating the median value for the highest category in each study, where a value 20% in excess of the lower cut-point was assigned in lieu of a median value.

Pre-morbid diet and breast cancer survival

Six prospective cohort studies examined pre-morbid dietary consumption of antioxidant micronutrients and risk of breast cancer mortality (Table 1). Four of these reports were conducted in the US or Canada, and two others analyzed breast cancer cohorts in Australia. Whereas diet was assessed using similar methods (Food Frequency Questionnaire), the analysis and reporting of vitamin C consumption differed across reports (e.g. use of continuous versus categorical intake values). A continuous variable for pre-morbid vitamin C consumption was used in two studies, in which a relative risk estimate was reported per 100mg/day increase [28,29]. The cut-points for the highest categories of consumption were similar (>210 and >233 mg/day) in two reports that used categorical consumption of vitamin C [11,32]. The two remaining studies did not report values or cut-points for the highest levels of vitamin C consumption [30,33]. These six studies are described below.

The association between self-reported dietary intake of antioxidants at diagnosis and survival from breast cancer was studied in a population-based cohort of 451 breast cancer patients in Australia [32]. Women with breast cancer were enrolled between 1982 and 1984 and completed a 179-item self-administered FFQ. Follow-up (median of 5.5 years) was completed in

1989 through computerized record linkage to determine vital status, during which 112 (25%) of the cases died of breast cancer. This study examined pre-morbid diet and risk of breast cancer survival and did not take into account possible changes in diet after diagnosis.

For the highest levels of vitamin C intake (>234 mg/day), a tendency towards a protective effect was observed after adjustment for energy and other factors (RR=0.74, 95% CI, 0.42-1.30). However, this result was not statistically significant. The estimates for the second highest category of vitamin C intake (145-233 mg/day) did achieve statistical significance (RR=0.50, 95% CI, 0.27-0.91). Each quartile of vitamin C intake, when compared with the reference category (< 71mg/day) showed an inverse relationship with risk of breast cancer related death. Even though the relative risk estimates indicated a slight to moderate protective effect with increasing categories of intake, no statistically significant trend for increasing dose of vitamin C and risk was observed (P=0.14). This reflects the finding of the strongest protective effect observed in the second highest (145 – 233 mg/day) category.

The high participation rate of 81% among cases (451 of 559 eligible cases) enhanced the studies internal validity and little difference between included and non-included cases was observed with respect to sociodemographic and tumor characteristics. The authors also reported that cases were interviewed as soon as possible after diagnosis to minimize the potential that disease status may have influenced pre-morbid diet classification. Overall in this report, modest evidence linking dietary vitamin C intake and breast cancer death was observed.

A second Australian cohort study of 193 women with breast cancer was conducted in the mid-1980's [30]. Women were interviewed and instructed to complete a self-reported FFQ in their own time. With a maximum of 6 years of follow-up, significantly fewer deaths were observed among the highest level of vitamin C consumption. In this study, three deaths were reported among the highest, 7 in the intermediate and 11 in the lowest tertile of vitamin C consumption (P for trend=0.03). Whereas women in the highest consumption level of vitamin C showed greater disease-free survival, the limited number of observed deaths precludes any definitive conclusions.

A Canadian study [11] found pre-morbid dietary consumption of vitamin C to be preventatively associated with breast cancer related mortality. In this study from the National Breast Screening Study in Canada (NBSS), pre-morbid dietary data on 678 breast cancer cases was analyzed. Seventy-six deaths due to breast cancer were identified during the course of three to six years of follow-up time. An 86-item dietary history questionnaire was used to assess dietary consumption of foods and supplements in the previous month. Only women who completed the questionnaire prior to the diagnosis of their breast cancer were included in this follow-up cohort. The authors observed a significant trend for each 100mg increase in vitamin C consumption when analyzed as continues values of intake. In categorical analyses, the highest level (> 210 mg/day) was associated with a reduced risk of dying from breast cancer (RR=0.43, 95% CI, 0.21-0.86), and a monotonic reduction in risk was reported for each increasing quartile.

The effect of vitamin supplementation was investigated among 100 women in this study who reported the use of vitamin C supplements. A non-significant slight reduced risk was observed among the vitamin C supplement consumers (RR=0.88, 95% CI, 0.42-1.82) compared with non-users of vitamin C supplements.

Among these women, a trend was also observed for each 10mg increase in vitamin E from diet. The highest category of vitamin E intake (>24 mg/day) compared with the lowest (<14 mg/day) showed modest evidence for a reduced risk (RR=0.55, 95% CI, 0.26-1.17).

A concern in this report was the fact that of the 1270 breast cancer cases identified only 678 completed a diet questionnaire. The authors report that non-participants were slightly less educated and more likely to smoke, factors that may influence dietary habits, particularly the consumption of fruits and vegetables [34,35]. As a result, selection bias may have been introduced, in which the distribution of fruit and vegetable consumption may have been shifted towards higher intakes, and thus not be representative of the levels consumed among all cases. It is unclear what effect if any this would have on the relative risk estimates.

The Iowa Women's Health Study has provided prospective data on a uniquely homogenous and stable population. This population consists of predominantly white women between the ages of 55 and 69 years living in rural communities in the Midwestern US. Zhang and colleagues [33] identified 698 postmenopausal breast cancer cases among 41,837 women between 1986 and 1991. Diet was assessed before the onset of breast cancer (January 1986) with a semi-quantitative FFQ, and did not account for changes in dietary behavior after diagnosis. The follow-up period after diagnosis consisted of a maximum of 6 years and a minimum of only one-year, with a median follow-up of 2.9 years. A total of 40 women died with a cause attributed to cancer on their death certificates. As there were only 40 cases identified with the outcome of breast cancer-related death, limited power was available to detect even modest associations. Information on breast cancer treatment or recurrence was not available. The authors reported no significant associations between antioxidant micronutrients from diet and breast cancer survival, although no specific data regarding antioxidants were presented.

A small prospective study from Michigan examined the potential effects of diet on breast cancer prognosis, both recurrence and survival [29]. A total of 149 women with primary breast cancer completed a 100-item FFQ [36], which was designed to assess usual diet in the first year prior to diagnosis. Data obtained from the University of Michigan Tumor Registry identified 28 recurrences and 26 deaths. The recurrences were analyzed together (local, regional and distant) and deaths, whether breast cancer-related or due to any cause, were also analyzed together. Two separate analyses were then conducted, one for recurrence and one for overall death. Cox proportional hazard models, which measure rate ratios, were used to examine the relationship between dietary antioxidants and risk of recurrence or death. What percentage of deaths in this population was related to breast cancer remains uncertain. The authors made no mention if a woman could have been counted as both a recurrence and subsequently as a death, if recurrence led to death a women could have been counted twice [37]. Perhaps this could explain the similar results observed for vitamin C in each of these analyses.

Results indicate that for each 100 mg increment in vitamin C consumption, no associations with risk of breast cancer recurrence (RR=1.03; 95% CI, 0.69-1.54) and for death (RR=0.92; 95% CI, 0.57-1.47) were observed. The authors used continuous variables for their dietary exposures. Vitamin C was assessed per 100 mg increase in intake. The authors made no mention for whether a monotonic relationship existed between vitamin C consumption and risk of either recurrence or death, which would determine whether use of a continuous variable is appropriate. In the quantification of total vitamin C intake, supplements were not included. This omission may have led to misclassification of exposure, since supplements typically account for a substantial proportion of total vitamin C intake in a population [38]. Despite this, perhaps the most limiting component of this study was its sample size, which precluded the ability to categorize vitamin C and examine high levels of intake.

Another concern with this report, as discussed earlier, was the use of pre-diagnosis measurement of diet. Women may possibly have changed their diet after diagnosis, either as a

consequence of their disease and treatment or to reduce their risk of recurrence and adverse outcomes. A change in diet will have altered the categorization of antioxidant micronutrient exposure. For example, a woman with low consumption prior to diagnosis may alter her diet after diagnosis to reflect increased concern for her physical health, with higher consumption of antioxidant-rich fruits and vegetables. In contrast, women with adequate or better consumption of antioxidants may have been diagnosed with a more advanced disease, which required extensive chemotherapy or radiation. These treatments have been shown to reduce appetites and alter dietary consumption of micronutrients [39]. With the exception of Holm et al. [25], no other studies included in this report examined vitamin intake with regards to cancer treatments, though many did control for disease stage.

The calculation of antioxidant vitamin C consumption without the use of supplements may have influenced the results. Particularly since vitamin C supplements can account for the large proportion of total vitamin C intake. Consumers of high levels of fruits and vegetables have been shown to be more likely to use an antioxidant supplements [40]. Whereas including supplements may have increased the variance of intake, it is unclear whether this could have significantly altered the vitamin categorization of cases and controls.

The question of whether reduction in risk was a function of absolute differences in vitamin C intake among the highest categories of consumption was examined. A graphic (Figure 1) overview of these aforementioned studies shows the relative risk estimates plotted against increasing doses for pre-morbid vitamin C intake and breast cancer survival. Four of the six reports described in this section provided vitamin C dose information in mg/ day and the corresponding RR estimates. Across these four reports, visual inspection of the RR estimates indicates little to no trend with increasing levels of consumption. Although the upper confidence limits were greater than 1.0 for most of the estimates, each median value for reported categories of consumption had a corresponding RR estimate less than 1.0.

The lowest cut-point for a non-reference category, was 100 g/day, and the median value for the highest category of vitamin C was 280 mg/day. Based on the latest dietary reference intake report [13] for women, the range in values reported here are all above the minimum cut-point of vitamin C (90 mg/day) thought to be threshold for disease prevention, antioxidant protection and health promotion. This Institute of Medicine [13] report did not discuss a dose response relationship. Dose response relationships for vitamin C and breast cancer incidence have not been consistently reported, when they have been discussed no trend has been observed [5,6,8,41]. However, results for doses in excess of 100 mg/day have, for the most part, been associated with reduced risks of breast cancer incidence [7].

Pre-morbid diet and breast cancer recurrence

Three studies reported results for antioxidant consumption prior to diagnosis and subsequent risk of breast cancer recurrence (Table 1). Two of these reports [28,29] were discussed in the previous section as they reported results for both survival and recurrence. While Saxe et al. [28] and Hebert et al. [29] studied U.S. breast cancer cohorts and reported vitamin C per 100mg/day increase, Holm and colleagues [25] utilized a Swedish cohort and reported energy-adjusted (mg per megajoules of energy) results for increasing vitamin E and β-carotene.

Disease-free survival and breast cancer recurrence was investigated in a study of 240 predominantly post-menopausal (87%) women with early stage breast cancer in Sweden [25]. Within four months of diagnosis and subsequent to cancer treatment, the women completed a dietary history interview that dealt with dietary habits during the past year before diagnosis.

Four years of follow-up were used to assess recurrence, defined as a loco-regional recurrence or distant metastasis. Fifty-two (22%) patients had a breast cancer recurrence during follow-up.

Vitamin E was positively associated with treatment failure, where the RR was 1.19 (95% CI, 1.03-1.37) for each milligram per MJ increase. However, vitamin E was strongly correlated with total fat intake, which was the strongest predictor of failure. The independent result for vitamin E was not adjusted for total fat intake. The distribution of vitamin E consumption followed a similar trend, where higher consumption was reported among women who failed treatment in the first two years of diagnosis. These women reported a mean intake of 12.2 mg/ 10 MJ compared with 9.9 mg/ 10 MJ among disease-free survivors ($p=0.02$).

Women in the failure category also reported the lowest intake of vitamin C ($P=0.03$). The mean intake of vitamin C among disease-free women was 113.4 mg/10 MJ, whereas the mean intake among women with a recurrence was 102.0 mg/10 MJ. The same pattern, 5.0 mg/10 MJ compared with 4.1 mg/10 MJ was also shown by β-carotene.

Potential weaknesses in this study include the fact that the specific treatments received were unspecified in this study and may have differed by dietary status. Women with diets higher in fruits and vegetables, or supplements users, may be more likely to have characteristics that are associated with receiving cancer treatments (e.g., chemotherapy). Differences in treatment received could have led to significant confounding, since treatment may be the strongest predictor of disease-free survival. Whether this would have explained the differences between groups is unclear. The energy adjustment method utilized in this study could have introduced systematic error. Vitamin C is not highly correlated with energy intake [42], and often over-reported by self-reported dietary assessment tools as opposed to fat intake [43]. Energy adjustment, it can be argued, may be inappropriate for micronutrient vitamins [42]. Furthermore, the reporting of vitamin C in mg/ MJ does not offer easy interpretation for public health recommendations.

Post-diagnosis diet and breast cancer recurrence and survival

Only one study [28] accounted for changes in dietary intakes after diagnosis of breast cancer, and another [31] assessed solely post-diagnosis diet. Diet and body weight in relation to breast cancer recurrence and survival were examined among 472 women diagnosed with early stage breast cancer between 1982-1984 [28]. The population was enrolled at Memorial Sloan-Kettering Cancer Center in New York. This tertiary cancer center tends to receive patients from higher socioeconomic strata, who may have diets different than the general source population. Interviews were conducted at the time of diagnosis and again at two years into the follow-up period. A 34-item FFQ was used to assess diet at diagnosis and at two years post-diagnosis. A shortened FFQ, such as this, serves to identify major food groups, but may underestimate total energy consumption. To combat this brief FFQ, the authors sought to energy-adjust based on estimates from NHANES.

Whereas energy adjustment can influence the results of dietary factors for breast cancer [44], what effect this external adjustment method would have for micronutrients as opposed to fat intake is unclear [42]. As opposed to the underestimation of fat intake in the FFQ, micronutrients may in fact be overestimated [45], and energy adjustment may lead to a disproportionate inflation of micronutrient intakes. Since this was a shortened 34-item FFQ, and designed primarily to capture sources of fat, measurement error could have strongly influenced the assessment of vitamin C intake. Vitamin C intake may have been underestimated if supplements were not included in the questionnaire, but it is unclear whether they were or not.

The maximum follow-up for each case ranged from eight to 10 years. A total of 109 (23%) recurrences were identified, and 73 (15%) women died from their breast cancer. In order to examine the association between antioxidant micronutrients (vitamin C) from diet and breast cancer recurrence, the authors used a proportional hazards model. No data was reported for dietary intake of vitamin C at the time of diagnosis and subsequent risk of recurrence or death, and limited results for change in vitamin C intake at the second interview were reported. About 20% of women were missing data for the interval after baseline data was obtained. The variable for vitamin C was computed as the change in vitamin C consumption between the baseline and the follow-up questionnaire. Approximately a 40% reduced risk of recurrence was observed for each 100mg increase (positive change) of vitamin C from foods reported after diagnosis (RR=0.57, p=0.11). The authors also found a non-significant decreased risk of recurrence and death for each 100mg increase (positive change) in vitamin C consumption from food sources (RR=0.48, p=0.14).

One important feature of this study was the ability to focus on dietary changes made after women were diagnosed with breast cancer. However, the second FFQ used to assess post-diagnosis diet was missing for 20% of the women. The authors conceded that these data were not missing at random, and a high proportion of women with recurrences did not complete the second FFQ. This may have resulted in a selection bias if a differential distribution of vitamin C consumption between cases and controls was present.

The Nurses Health Study routinely publishes results for dietary factors and cancer, and has become known as one of the more reliable cancer cohorts. In their report on dietary factors and breast cancer survival, Holmes and colleagues [31] chose to emphasize diet after diagnosis since it offers the possibility to advise dietary modifications for women already diagnosed with the disease. The Nurses' cohort consists of 121,700 female registered nurses followed since 1976. Between 1976 and 1990, 3,041 (2.5%) women were diagnosed with invasive breast cancer. These women were followed for a median time period of 157 months, and the median time between diagnosis and diet assessment was 24 months. For their analysis however, 569 women without dietary assessment after diagnosis and an additional 490 women without adequate tumor staging information were excluded. In total, 1,059 (35%) breast cancer cases were excluded from the analysis.

Of the 378 patients who died during the follow-up period, 326 (86%) died as a result of breast cancer. The authors used a validated FFQ to assess post-diagnosis intakes of 83 nutrients. Among the nearly one hundred food items analyzed, the results for vitamins C and E with and without supplements were reported in quantiles. Unfortunately, quantitative cut-points were not reported and as a result, the highest quantile becomes less interpretable. Nonetheless, the highest intakes of vitamin C and E from diet alone showed modest evidence for a protective effect, where RR estimates equaled 0.80 (95% CI, 0.0.58-1.10) and 0.82 (95% CI, 0.60-1.12), respectively. When supplements were included, the modest protective effect for vitamin C disappeared (RR=1.18, 95% CI, 0.55-0.85-1.63), but vitamin E maintained its protective association (RR=0.77, 95% CI, 0.56-1.05).

Whereas the analysis did control for numerous factors, several limitations were apparent in this study. First, the exclusion of more than one-third of breast cancer cases may have introduced a selection bias. Although it may have been pertinent to exclude women without post-diagnosis dietary assessment, the removal of 490 women without sufficient tumor staging may have been inappropriate. An analysis should have been run with these women included to examine whether the results would have been influenced. If disease stage were associated

independently with vitamin C or E consumption, the inclusion of these women would have changed the results only if the excluded women had disproportionately high or low stages compared with included cases. The authors mention that in modeling attempts to control for disease stage more precisely, the results remain unchanged. Secondly, in accordance with the majority of reports described previously, information on breast cancer treatment was not included. Finally, analyses of more than one-hundred food items may have led to spurious associations, and no attempt to adjust for multiple comparisons was reported.

The effect of diet on breast cancer survival is likely to be a complex combination of pre- and post-diagnosis consumption. For a woman facing a diagnosis of breast cancer, it is only diet after diagnosis that can be changed. Regardless of the limitations in these two reports, an assessment of post-diagnosis diet may be most appropriate with regards to prognostic outcomes. However, with only two published reports, one analyzing treatment failure and the other breast cancer death, no conclusion can be drawn at this point for either vitamin C or E intake after diagnosis.

Descriptive Studies

Only one descriptive study reported on the association between vitamin C and breast cancer mortality. An ecological study in China [24] examined over 3000 women in multiple rural counties in 1983. A 1973-1975 nationwide survey was used to determine breast cancer mortality rates stratified by 5-year age groups and county. Fasting blood samples were taken and combined into age and sex-specific pools. The assumption that pooled samples reflects the average values for individual samples were verified in laboratory comparisons. A structured questionnaire was used to obtain demographic and dietary data. The details for the dietary assessment tool were not discussed. Nevertheless, an inverse correlation was identified between vitamin C (mg/dL) from serum and breast cancer mortality ($R = -0.26$, $P<0.05$), which supports the hypothesis that areas with highest vitamin C consumption were regions with the lowest breast cancer mortality rates.

This study examined average serum levels across populations, not individual levels, and the temporal sequence of cause and effect was not accounted for; thus, causal inferences cannot be drawn. Whereas mortality rates were observed in 1973-1975, these were compared with exposure status determined 10 years later. The temporal sequence between exposure and disease was reversed (rates were assessed 10 years prior to exposure assessment), and survival rates may possibly have changed over the years, or dietary patterns themselves may have changed.

Intervention Studies

Two non-randomized intervention studies [22,23], limited with small sample sizes and lack of an appropriate control groups, examined vitamin C supplementation and breast cancer prognosis. Each of these trials consisted of less than 35 women. As a result, these trials contributed little to the overall assessment of high intakes of vitamin C on breast cancer recurrence and disease-related mortality, nor were they included in the meta-analysis.

The effect of long-term vitamin C supplementation (3 g/day) was studied in a small intervention trial [23] of 27 women with non-metastatic breast cancer. Ascorbic acid levels in blood rose significantly ($P < 0.001$) over three months time since diagnosis among supplemented cases when compared with non-supplemented cases. After five years no differences in serum vitamin C were observed between groups with regards to local recurrence, distant recurrence and mortality events. However, only four local recurrences were observed (3 controls, 1

supplemented case). Whether supplementation continued throughout the five-year follow-up period as evaluations ceased after approximately one year was not reported.

The other clinical trial to examine antioxidant supplements among women with breast cancer was the Adjuvant Nutritional Intervention in Cancer (ANICA) study in Denmark [22]. This small trial consisted of 32 patients with metastatic disease. Antioxidant supplements (Vitamin C: 2850 mg, Vitamin E: 2500 IU, β-carotene: 32.5 IU, selenium: 387 μg) in addition to essential fatty-acids and coenzyme Q10 were administered, on an adjuvant basis with cancer treatment, for up to 12 months. To determine compliance, vitamin biomarkers were assessed periodically, and significant increases in serum for each antioxidant were observed. The clinical outcomes of interest were tumor spread and survival. The authors observed no deaths during trial period, whereas four were expected. Furthermore, six patients were reported to have had a partial remission. This small study provides very limited support for beneficial effects of antioxidant supplements. The independent effect for total antioxidants used in this trial cannot be separated from the potential effects for the fatty-acids and/ or coenzyme Q10 supplements, since they were given in combination with one-another.

Meta-analysis

A meta-analysis was conducted using RR estimates for highest categories of pre-morbid vitamin C consumption and risk of breast cancer related mortality. The individual RR estimates for extreme contrasts across reports ranged from 0.40 to 0.92. The reference categories ranged from intakes less than 71 mg/day to less than 110 mg/day, whereas the highest categories ranged from greater than 100 mg/day to greater than 233 mg/day. The difference between the midrange dose of the highest consumption categories and the midrange dose of the reference groups was approximately 125 mg/day.

Based on the limited number of reports and differences in the categorization of vitamin C consumption (using a continuous versus categorical variable), a fixed effect relative risk estimate of 0.73 (95% CI, 0.59-0.90) and a random effects relative risk estimate of 0.72 (95% CI, 0.57-0.90) were computed for the highest reported category of consumption. No evidence for heterogeneity of risk estimates across the five reports was observed (p-value=0.38). This result suggests that high consumption of vitamin C decreased the risk of breast cancer-related mortality from 10% to as high as 43%, with a point estimate of an approximate 28% reduction. Three studies reported RR estimates for each 100mg/day increase in vitamin C consumption. The random effect relative risk estimate for these three reports was 0.65 (95% CI, 0.40-1.07), with no evidence for heterogeneity was observed (p-value=0.20). A 100mg per day increase in vitamin C consumption reduced risk of breast cancer mortality, though this result failed to achieve statistical significance at $p<0.05$.

One study [33] was not included in the meta-analysis because of the lack of quantitative results. They did write "no association for vitamins C or E". To estimate the influence of a result of no association from this report, a sensitivity meta-analysis was re-run with an additional result of 1.0 and an estimated standard error consistent with other results that were reported in this study (95% CI, 0.5 – 2.2). The fixed effect relative risk estimate was 0.71 (95% CI, 0.54-0.93), with no significant heterogeneity across studies (p=0.43), and the random effect relative risk estimate was 0.74 (95% CI, 0.61-0.92). Even with the inclusions of the Zhang et al. [33] report, the protective effect for vitamin C remained significant. The minimal effect on the fixed and random effects relative risk estimates, with the inclusion of Zhang et al. [33], indicated the large

standard error (stemming from the low number of breast cancer related mortality events) in that study's estimate.

Conclusions

The published epidemiologic evidence suggests that modest protection from breast-cancer related mortality is conferred by consumption of high pre-morbid intakes of vitamin C from diet. The mean dose of vitamin C was not reported in all the studies, and the minimum level of vitamin C necessary to elicit a protective effect could not be estimated, because quantitative cut-points were not used in all reports. In addition, the same cut-points for categories were not used across studies. Furthermore, one study [33] did not show results for vitamin C and survival, but did report that no association was observed. The inclusion of this study, by imputing a null result into the meta-analysis, attenuated the summary estimate, but this change was minimal.

Eight epidemiologic cohort studies assessed dietary consumption of antioxidants, six prior to diagnosis and two post-diagnosis. Among these reports, results for vitamin C intake were most commonly reported. All published relative risk estimates for the risk of breast cancer related mortality or recurrences with highest levels of vitamin C intake from diet were on the protective side of the null value (< 1.0). Statistical significance at the level of $p<0.05$ was achieved in four of these studies [11,25,30,32].

Vitamin E showed less consistency of results. Slight protection was reported for the highest levels of consumption in two studies, no association in another two, and a slight increased risk in one report. The major sources for vitamin E are nuts and oils. Oils are highly correlated with fat intake, which has been associated with decreased breast cancer survival [29,33,46,47]. The association between vitamin E and breast cancer mortality could possibly be explained by the inadequate control for dietary fat intake or the inability to separate the effects for vitamin E and fat intake.

Based on the available epidemiologic studies, vitamin C consumption appears to exhibit some consistency of results. The potential protective effect may be quite modest for pre-morbid vitamin C consumption and may not be identified without large sample sizes. Publication bias may have contributed to the overall assessment of these micronutrients. Since only a limited number of reports have examined any component of diet and breast cancer prognosis, publication bias is less likely.

These studies suggest that more attention and further studies should address pre-morbid dietary habits in relation to the prognostic outcomes of breast cancer. However, to control for the effect of post-diagnosis dietary modifications on the clinical course of the disease, further studies need to be conducted with full ascertainment of dietary changes prior to and subsequent to diagnosis. Even though women who report high pre-diagnostic intakes of antioxidants from diet and supplements tend to continue this high intake into the post-diagnosis period, assessment of pre and post-diagnosis intake is necessary to reduce measurement error [40].

An alternative hypothesis must also be considered, which may explain away some of the association between vitamin C and breast cancer survival. Epidemiologic evidence supports a protective effect for total fruit and vegetable consumption in breast cancer prevention [48]. Fruits and vegetables are the main dietary sources for vitamin C and the carotenoids. Whereas these studies did energy adjust their estimates, none controlled for total fruits and vegetable consumption. Therefore the independent effects of vitamin C cannot be separated from the

effects of higher consumption of fruits and vegetables. Despite the limitations and alternative hypotheses, vitamin C showed limited, but modest evidence for a protective role in breast cancer prognosis. Further research examining pre-morbid and post-diagnosis intake of antioxidants is warranted.

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Table 1: Cohort studies of pre-morbid intake of antioxidant micronutrients from diet and/ or supplements and risk breast cancer survival.

Reference: Country	*N*	*Follow-up (years)*	*Exposure measure*	*Intake*	*Outcome*	*RR*	*95% CI*
Saxe, 1999 USA	149	5 (median)	Vitamin C	Per 100 mg/day	Survival	0.92	0.57-1.47
				Mean=161 mg/d	Recurrence	0.96	0.69-1.19
				Per 2000 mcg/day			
			β-Carotene	Mean=3543	Survival	0.91	0.69-1.20
				mcg/ day	Recurrence	1.03	0.69-1.54
Holmes, 1999 USA	1982	13.3 (median)	Vitamin C	Quantiles 2	Survival	0.66	0.46-0.94
				3		0.86	0.61-1.20
				4		0.80	0.57-1.12
				5		0.80	0.58-1.10
			Vitamin E	Quantiles 2	Survival	0.67	0.48-0.94
				3		0.68	0.49-0.95
				4		0.66	0.48-0.91
				5		0.82	0.60-1.12
Hebert, 1998 USA	472	8-10	Vitamin C	Per 100 mg/day	Survival	0.57	P=0.11
					Recurrence	0.48	P=0.14
Zhang, 1995 USA	698	≤ 6	Vitamin C Vitamin E β-Carotene	Not reported	Survival	No Associations (data not shown) RR range 0.6-1.3 (P>0.24)	
Jain, 1994 Canada	678	≤ 6	Vitamin C	<110 mg/day	Survival	1.00	
				110-156		0.58	0.32-1.05
				156-210		0.57	0.31-1.06
				>210		0.43	0.21-0.86
				Per 100 mg/day		0.67	0.48-0.93
				Supplements		0.88	0.42-1.82
			Vitamin E	13.7-18.6 mg/day		0.68	0.37-1.27
				18.6-24.3		0.61	0.31-1.20
				>24.3		0.55	0.26-1.17
				Per 10 mg/day		0.72	0.51-1.02
			β-Carotene	>7,690 mcg/day		0.43	0.23-0.99
				Per 2000mcg/day		0.85	0.72-0.99
Rohan, 1993 Australia	412	5.5 (median)	Vitamin C	<71 mg/day	Survival	1.00	
				72-110		0.59	0.33-1.07
				111-144		0.80	0.47-1.38
				145-233		0.50	0.27-0.91
				>233		0.74	0.42-1.30
			β-Carotene	>8,058 mcg/day		0.68	0.36-1.27
Ingram, 1993 Australia	103	≤ 6	Vitamin C	Highest tertile	Survival	0.4	P=0.03
			Vitamin E	Highest tertile		0.7	P=0.41
Holm, 1993 Sweden	240	≤ 4	Vitamin C	Mean daily intake	Recurrence	P=0.003	
			Vitamin E	Mean daily intake		No Association	
			Vitamin E	Per 1mg/ 10 MJE		1.19 (1.03-1.25)	
			β-Carotene	Per 1mg/MJE		No Association	

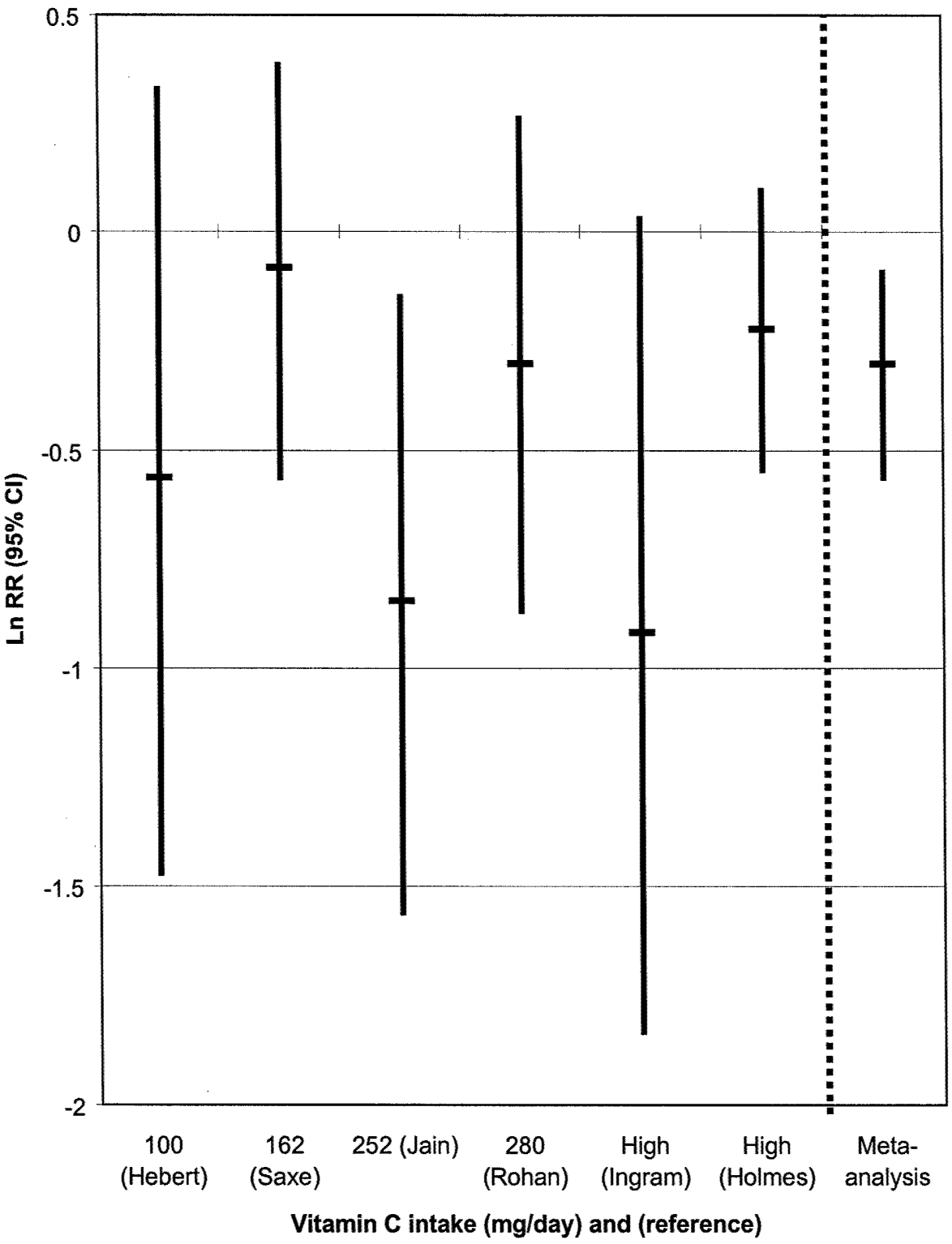
Abbreviations: RR, Relative Risk Estimate; CI, Confidence Interval; BMI, Body Mass Index, ER, Estrogen Receptor; PR, Progestin Receptor; SES, Socioeconomic status

Table 2. Intervention studies examining antioxidant supplements and breast cancer prognosis.

Reference:	*N*	*Antioxidant*	*Intake*	*Outcome*	*Comments*
Lockwood, 1994 Denmark	32	Vitamins C, E, β-carotene, selenium	C (2500mg), E (2500 IU), β-carotene (32.5 IU), selenium (387 mcg)	0 deaths observed/ 4 expected. PR in 6 patients	Non-randomized. No control group.
Poulter, 1984	27	Vitamin C	3 g/day	No differences in 5-year survival.	Randomized, controlled.

Abbreviations: IU, International Units; PR, Partial Remission

Figure 1. Relative risk estimates and 95% confidence intervals for breast cancer survival with increasing dose of pre-morbid vitamin C intake.



Change in antioxidant supplement use after diagnosis and factors associated with use among early-stage postmenopausal breast cancer patients.

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Support: This work is supported by a dissertation grant from US Department of Defense Breast Cancer Research Program (DAMD-17-01-1-0350).

Acknowledgements: The authors acknowledge Drs. Robert Millikan, John Anderson and Michael Symons for their review of this manuscript.

Shortened Title: Antioxidant supplements and breast cancer

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Abstract

Background: Dietary supplement and medicinal herb use is more prevalent among breast cancer patients than among the general US population. However, few studies have quantified the change after diagnosis and identified the characteristics of supplement use among postmenopausal breast cancer patients.
Methods: We re-contacted 385 women diagnosed with breast cancer between 1986 and 1988 enrolled into a case-control study on diet and cancer with a questionnaire to ascertain use of nutritional supplements and medicinal herb products before and during 12 to 14 years of post-diagnosis follow-up time.
Results: One or more supplements or medicinal herbs were used by 80.5% of women. Use of supplements increased significantly after diagnosis. Antioxidant supplement use (vitamin C, E, beta-carotene, selenium or an antioxidant combination) increased from 34% pre-diagnosis to 56% after diagnosis, and was overall used by 64% of this cohort at some time during their adult life. Current exercise (OR=2.62, 95% CI=1.2-5.9) and concurrent use of medicinal herbs were positively associated with new use of antioxidant supplements after diagnosis. While increasing age was associated with supplement use prior to diagnosis, and opposite direction was observed post-diagnosis.
Discussion: Antioxidant, vitamin and mineral, and medicinal herb supplement use were highly prevalent among women with breast cancer, more so than in the general population. The increase in use of antioxidant supplements and medicinal herbs after diagnosis of breast cancer is a public health concern because of adverse effects and uncertain efficacy.
Key words: Antioxidants, Nutritional Supplements, Medicinal Herbs, and Breast Cancer.

Introduction

As breast cancer survival rates improve[1], women with breast cancer seek to optimize their quality of life and prevent disease recurrence. National surveys have shown that about 46% of US adults use vitamin or mineral supplements and 14% report using a medicinal herb product[2]. Breast cancer diagnosis is associated with a significant increase in nutritional supplement and medicinal herb use[3]. Among women with breast cancer, use of nutritional supplements and medicinal herbs have been found to be as high as 81% and 21%, respectively, and as much as 50% greater than the general population[3-6].

The length of time since diagnosis and demographic characteristics such as older age, higher income and education, and consumption of a more vegetable rich diet have been shown to be predictors of supplement use among breast cancer survivors[3,5-7]. Previous studies have not examined use of specific supplements and medicinal herb types, nor have they examined use over time since diagnosis. In this study, we examined use of supplements and medicinal herbs, particularly antioxidant supplements, among post-menopausal breast cancer patients in 12 to 14 years of follow-up time since diagnosis. We further identified characteristics associated with antioxidant supplementation, and quantified the change in use of specific types of supplements after diagnosis of breast cancer.

Methods

Subjects: Participants with a diagnosis of invasive breast cancer were recruited from five Boston area hospitals between 1986 and 1988. A total of 407 postmenopausal breast cancer cases were enrolled into a population-based case-control study [8]. Cases were post-menopausal at diagnosis, with no history of prior cancer other than non-melanoma skin cancer. Early stage, non-metastatic breast cancer cases (AJCC stage I, II) were recruited, however, 27% of this population was later found to have positive lymph nodes, and re-classified as stage IIIB. Cases have been followed since diagnosis as part of the FASTCAB study (Fatty Acid Stores Tumor Characteristics and Breast Cancer) where additional vital status, adipose, tumor block and medical record information have been collected.

A first round of questionnaires was mailed in November 1999, with follow-up mailings to non-respondents in January 2000, to the 393 women from the FASTCAB population who had not refused further participation. Next of kin of cases who had died (N=123) during the follow-up period (1989 – 1999) received a proxy questionnaire.

The response rate was 65% for living cases and 38% for proxy respondents of cases who had died during the follow-up period. Of 270 living cases, 22 (8.1%) did not receive questionnaires due to incorrect or non-forwarding address information and were considered lost to follow-up, 10 (2.7%) refused participation and 64 (23.7%) were non-respondents after three consecutive mailings and phone contacts. Of the 122 next of kin identified, 23 (18.9%) did not receive questionnaires due to incorrect addresses and were considered lost to follow-up, 11 (9%) refused participation and 42 (34.4%) were non-respondents.

Supplement assessment: The questionnaire was a self-administered survey and took approximately 15 minutes to complete in focus group testing. There are two forms to the questionnaire. One is for living cases, while the other is for next of kin (NOK) of the cases who have died. The NOK questionnaire differs from the living case version in the style of question wording and the omission of current age and weight.

The questionnaire consists of 10 pre-coded closed response questions, a list of supplements (three categories: herbals, vitamins and minerals, and nutritional supplements) and 10 lines to insert the name of the supplement(s) selected from the list and answer questions regarding each supplement selected. The pre-coded questions concern the use of hormone replacement therapy (HRT), Tamoxifen, alcohol, smoking and consumption of soy containing foods. Classifications included never, past, and current use. For past and current use, we assessed the length of time of usage with approximate stop or start dates, respectively. Two questions were designed to verify breast cancer recurrence data obtained from medical records.

Whether the supplement was used before, after, or both before and after diagnosis of breast cancer, the total length of time of use in years, frequency per month and the approximate or average dose using the appropriate units (e.g., mg or IU) was assessed. Any use of supplements was defined by supplement use reported as before diagnosis, only after diagnosis or both before and after diagnosis. Use of supplements only after diagnosis was considered "new use".

Supplements were categorized in accordance with previous literature [5] (Newman, 1998). Antioxidants are defined as containing primarily antioxidant nutrients, including 1 or more of the following: β-carotene, vitamin C, vitamin E and selenium. Multivitamins are defined as containing a variety of vitamin and minerals, primarily one or more vitamins in combination with one or more mineral. Herbal products are defined as containing primarily one or more herbal or botanical product. Nutritional supplements are defined as formulations containing a nutrient or natural product that do not fall into an aforementioned category. Examples include fish oils, shark cartilage and coenzyme Q-10.

A 124-item Willett food frequency questionnaire (FFQ) was used as part of the original case control study (1986-1988). Supplement use was assessed by this FFQ for the year prior to diagnosis of breast cancer. From this FFQ, vitamin C, E, beta-carotene, selenium and calcium supplement use was obtained, and used to assess pre-diagnostic intake. Thus, two different questionnaires were used to assess supplement intake. The FFQ was used to assess pre-diagnostic use, whereas the follow-up questionnaire was used to assess post-diagnostic use of these supplements.

Statistical analysis: Descriptive characteristics at the time of study enrollment were compared, using chi square statistics, among respondents to the follow-up questionnaire and non-respondents. Use of supplements during the follow-up period was assessed for cases who responded to the follow-up questionnaire. Prevalence of use across supplement types and categories were summarized and examined, and the distribution and number of dietary supplement formulations and combinations used per participant were summarized.

Associations between dietary antioxidant supplement use and demographic characteristics (age, SES), personal characteristics (BMI, smoking, alcohol, exercise, parity, oral contraceptive use, hormone replacement therapy and ages at first pregnancy, menarche and menopause) and cancer-related characteristics (AJCC tumor stage, radiation therapy and use of Tamoxifen) were analyzed by calculating chi square statistics in bivariate models. For continuous variables, such as age, age at menarche, first-pregnancy and menopause, BMI, and caloric intake, T-tests were used to compare mean values between users and non-users. Antioxidant supplement intake was defined as use of vitamin C, E, beta-carotene, selenium or formulas that contain antioxidant combinations. Logistic regression analysis was performed, using a single category for antioxidant supplement intake (ever versus never) as the dependent variable. Logistic regression models were used to determine the association between each independent variable and use of antioxidant supplements while controlling for all other independent variables.

Results

Table 1 shows demographic characteristics of cases stratified by response status. The mean age of cases (N=407) at diagnosis was 64 years (range, 47 – 93). Participating cases in this follow-up study were younger than non-respondents: 62.1 years compared with 66.3 years of age at diagnosis (p=0.001). Using data from the FFQ, again no

difference between respondents and non-respondents was observed for vitamin C, E, or calcium supplement use prior to diagnosis.

Frequency of Supplement Use:

One or more supplements were used at some time by 80.5% of women. Among those who took supplements, 67% used more than 1 supplement and 24% took 5 or more. The mean number of supplements taken was 3.9 (range, 1-10), with 6.5% of women consuming 10 different supplement formulations. Table 2a shows the use of antioxidant supplements stratified by pre-diagnostic and post-diagnostic use, which was further sub-divided into new use and continued use (both pre and post diagnosis use). Sixty-two percent of this cohort used an antioxidant supplement (Vitamin C, E, Beta-carotene, Selenium or an Antioxidant Combination) at some time during their adult life. Among women who reported any use of an antioxidant supplement, more than 45% of these women began use of antioxidant supplements specifically after their diagnosis. Aside from use of multivitamins (62%), calcium (50%) was the most commonly used supplement, followed by vitamin E (50%), and vitamin C (44%). Table 2b presents the frequency of nutritional supplement and medicinal herb use at any time and new use after diagnosis. Individual medicinal herbs were relatively uncommon among these women, but 23% reported use of at least one type of medicinal herb. Garlic, Echinacea, chamomile, and ginkgo biloba were the most common. Most medicinal herb use was begun after diagnosis of breast cancer.

Comparing pre-diagnosis use of vitamin and mineral supplements by cases with that of controls from the original case-control study shows similar frequencies of use. Vitamin C supplements were used by 24.8% of cases and 27.3% controls, and vitamin E supplements were used by 20.2% of cases and 23.7% of controls. In general, about a 3% increase in use among the population controls was observed. The observed differences in use between cases and controls did not approach statistical significance (data not shown).

Duration of use and frequency per week was assessed for each antioxidant supplement. Of respondents who reported total years of use, Vitamin C supplements were used, on average, for 8.7 years (0.1-40 years), vitamin E supplements were used 6.9 (0.5-35 years) and multivitamins were used a mean of 12.4 years (0.5-60 years). Among those who supplied weekly frequency information, most users of vitamin C, E and multivitamins reported daily use (7 times per week).

Change in Supplement Use:

Transition into the post-diagnosis period was marked by increases in supplement use across all categories. Use of an antioxidant supplement increased from 30% to 51%, indicating a 70% increase in use among cases after diagnosis. Among individual antioxidant supplements, vitamin E supplementation showed the greatest increase, from 19% to 39%, and C showed a smaller increase, from 23% to 37%.

Factors Associated with Any Use of Antioxidant Supplements:

Table 3 shows the prevalence of antioxidant supplement use according to demographic, personal and cancer-related characteristics and the multivariate logistic regression results for these factors and their association with use of antioxidant supplements. Socioeconomic status was positively associated with likelihood of

supplement use, with women in the highest category more than three and half times more likely to use antioxidant supplements before or after breast cancer. Age also showed some relationship with use, though the relationship for each category and trend was not statistically significant. Women who used oral contraceptives and/ or medicinal herbs were more likely to have used antioxidant supplements.

Factors Associated with New Use After Diagnosis:

Factors associated with new use of an antioxidant supplement after diagnosis were examined. Of all characteristics that appeared to show some relationship with antioxidant supplement use after diagnosis (Table 3), only current exercisers (OR=2.62, 95% CI, 1.2-5.9) were significantly more likely to begin use of an antioxidant supplement after diagnosis (Table 3). Users of medicinal herbs were also more likely to use an antioxidant during the post-diagnosis period. Socioeconomic status did appear to show some relation to ever use of antioxidant supplements; it was not associated with use solely after diagnosis. Hormonal factors, such as ages at menarche, menopause and first pregnancy, parity and use of hormone replacement therapy were not associated with supplement use, nor were caffeine intake and smoking status. Increasing age, higher tumor stage, BMI and daily caloric intake showed suggestion for a reduced likelihood with use, though not significant.

Discussion

Despite the lack of evidence of any health or survival benefit in women with breast cancer, dietary supplement use was highly prevalent (80.5%) among this cohort of postmenopausal women diagnosed with breast cancer in the late 1980's. Multivitamins, vitamin E and vitamin C supplements as well as calcium were the most common products used during the study period. Antioxidant supplements accounted for a large proportion of total supplement use, nearly 64% of the total population reported use. These results are consistent with other studies, including Newman et al [5] who reported 80.9% use of nutrient and non-nutrient dietary supplements among breast cancer survivors, with highest intakes for vitamin E and C compounds (55% and 51%, respectively).

Use of all types of supplements increased significantly after diagnosis. More than 85% of the antioxidant supplement use was reported after diagnosis, and greater than half of the users reported new use after diagnosis. Results of this study indicate that use of antioxidant supplements increased from 34% pre-diagnosis to 56% after breast cancer. These results are consistent with Burstein and colleagues[3] who reported the use of mega-dose vitamin supplements among a cohort of 480 women diagnosed with early stage breast cancer to be 28.1 % after diagnosis compared with 10.6% use prior to their diagnosis. A recent study of breast cancer outpatients in the Midwest[4], found the consumption of vitamins and herbal medicines to be significantly greater among cases when compared with similar aged women in the population. Reported use of 80.5% is considerably higher than the 44% of women in the general US population who took dietary supplements in the Third National Health and Nutrition Examination Survey[9].

The women in this study were post-menopausal and predominantly white. Both of these characteristics have been associated with supplement use in other populations[10]. Among post-menopausal women in the general population, increasing age has been related to increasing vitamin and mineral supplement use [11].

Our study did not measure changes in use among the controls. As a result we were unable to observe secular changes in the target population which gave rise to the cases. The change in use we report from pre-diagnostic to post-diagnosis periods may be attributed to the increasing popularity of supplement use in the population in general. Comparisons with population controls at study entry in 1986 to 1988 shows that these cases had similar frequencies of use with the women from the source population. Among all white women in the 1992 National Health Interview Survey, only 25.1% reported any supplement use, and 8% and 4.6% used vitamin C or E, respectively[12]. More recently, dietary supplement use for females in the NHANES III study was 44% [9].

Among survivors, certain demographic, personal and disease-related characteristics were associated with use of antioxidant supplements. Current physical exercise, history of use of oral contraceptives and use of medicinal herbs were found to be most strongly related to supplement use after breast cancer diagnosis. Women in the oldest age group (70 +) were also the least likely to use an antioxidant supplement after diagnosis. This finding contrasted with the relationship between age and any use of an antioxidant (pre, post or both pre and post diagnosis), in which use was highest in the oldest age group. This result suggests that younger women were more likely to begin new use of antioxidant supplements after diagnosis.

Several limitations exist in this study. The survey focuses on past and current use of supplements. Cases may have to recall supplement use as much as 10-12 years earlier. Proxy respondents may be unable to accurately recount past usage of supplements for the person they represent. More than half of the proxy respondents were spouses, while the remainder were sons, daughters or siblings. Moderate to strong agreement for proxy questionnaire responses has been reported particularly when the proxy was spouse [13].

Self-report as opposed to in-person interview may also introduce measurement error. However, a study by Dorant and others,[14] found strong agreement between interview (gold standard) and a self-administered questionnaire regarding use of supplements (Kappa=0.69). The authors concluded that self-administered estimates of consumption of specific supplements may provide enough precision to correctly classify individuals as users or non-users of those supplements [14].

Use of a single questionnaire, versus multiple subsequent questionnaires, to examine long-term use may also introduce measurement error. Patterson and colleagues [15] found that estimates of current daily intake for supplemental micronutrients were roughly twice that of average daily intake over the past 10 years. Correlations between current intake and long-term intake from supplements alone were 0.77, 0.75, for vitamin C, vitamin E, respectively. However, for this study, classification of ever versus never may not have been influenced by the cumulative dose of supplement intake.

An alternative hypothesis for the increase in supplement use after diagnosis may simply be explained by a temporal increase in the general population over the past 10 years. Dietary supplements have increased in popularity within the past 10 years, when compared with the pre-diagnostic time period prior to 1986-1988. Nonetheless, it is evident that supplement intake in this population after diagnosis was considerably higher than estimates from women in the general US population. The high supplement use may as well be attributed to the influence of physical conditions, such as cancer, and the heightened concern for recovery [16].

The effects of antioxidant supplements among breast cancer patients on treatment outcomes, recurrence and survival have not yet been studied. Only limited epidemiological studies have investigated the association between antioxidants from diet and risk of breast cancer recurrence and mortality. Results have been inconsistent, although reduced risks of recurrence and mortality have been reported, without significant evidence for increased risk [17-22]. Negative interactions with chemotherapy and radiation therapy have been hypothesized [23]. However, the majority of *in vitro* studies demonstrated either enhanced effectiveness for antioxidants on chemotherapy or no effect [24,25].

In summary, based on this study, breast cancer patients have been turning to dietary supplements in much greater frequency then women in the general US population. Consistent with prior studies in breast cancer populations, the factors positively associated with antioxidant supplement use after diagnosis were current exercise, younger age, a BMI less than 25, and concurrent use of medicinal herbs. Unfortunately, the current state of scientific knowledge of supplements is considerably lagging behind the increasing frequency of use among cancer patients. Breast cancer survivors represent an increasing segment of the population for which limited evidence exists on the dietary supplements they are using in increasing frequencies.

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Table 1. Demographic characteristics at time of diagnosis or study entry of postmenopausal breast cancer cases by response to follow-up questionnaire.

Characteristic	Respondents[a] N=220	Non-respondents[a] N=187	p-value[b]
Age			
Mean (SD)	62.1 (7.8)	66.3 (8.7)	0.001
Deaths	47 (21.4)	78 (41.7)	0.001
SES (%)			
Low	13.7	13.4	
Medium	49.8	46.5	
High	36.5	40.1	0.75
BMI			
Mean (SD)	26.0 (4.8)	26.2 (4.4)	0.71
Smoking			
Past	33.3	38.0	0.33
Current	11.8	14.4	0.43
Use OC	21.9	16.6	0.18
Age at First Pregnancy			
Mean (SD)	26.0 (5.4)	25.9 (5.4)	0.55
Parity			
No children	14.6	21.0	
Children	85.4	79.0	0.09
Age at Menopause			
Mean (SD)	48.3 (6.0)	47.8 (6.0)	0.45
Age at Menarche			
Mean (SD)	12.7 (1.7)	12.9 (1.5)	0.39
Daily caloric intake			
Mean (SD)	1772 (625.6)	1897.1 (800.6)	0.11
Supplement Use[b]			
Vitamin C	23.2	26.7	0.41
Vitamin E	19.1	21.4	0.56
Calcium	22.3	25.1	0.50

[a] Values are percent of the population, unless indicated as mean values.

[b] Chi-square statistic used to compare distributions, T-test used to compare means.

Table 2a. Pre- and post-diagnosis use of antioxidant vitamin supplements among postmenopausal breast cancer cases (N=220).

Supplement	**Any Use**	**Use Pre-diagnosis**	**Use Post-diagnosis**		
	N (%) [b]	N (%)	N (%) New	N (%) Continued	N (%) Total
Antioxidant Type					
Vitamin C	97 (44)	51 (23)	40 (18)	41 (19)	81 (37)
Vitamin E	109 (50)	42 (19)	58 (26)	28 (13)	86 (39)
Beta-carotene	8 (4)	2 (1)	5 (2)	1 (0)	6 (3)
Selenium	10 (5)	4 (2)	6 (3)	2 (1)	8 (4)
Antioxidants NOS	13 (6)	0	13 (6)	0	13 (6)
Total Antioxidants[a] Use	**136 (62)**	**67 (30)**	**64 (29)**	**48 (22)**	**112 (51)**
Multivitamins	137 (62)	76 (35)	64 (29)	54 (25)	118 (54)

[a] Antioxidant: Vitamin C or E or Beta-carotene or Selenium supplements use, multivitamins not included in total antioxidant supplements.

[b] Missing values for pre versus post-diagnosis use may preclude summing sub-columns to 100% of any use. Values in parenthesis are percent of total respondents (N=220).

Table 2b. Use of vitamins and minerals, medicinal herbs and other supplements among post-menopausal breast cancer cases diagnosed between 1986 and 1988.

	Any Use	**New use post- diagnosis**
	N (%)[a]	N (%)[a]
Vitamins and Minerals		
Vitamin A	8 (4)	7 (3)
Vitamin D	4 (2)	4 (2)
B-complex	36 (16)	30 (14)
Calcium	114 (52)	71 (32)
Iron	3 (1)	2 (1)
Magnesium	17 (8)	15 (7)
Multiminerals	17 (8)	14 (6)
Folic acid	8 (4)	7 (3)
Medicinal Herbs		
Chamomile	17 (8)	14 (6)
Echinacea	16 (7)	15 (7)
Garlic	27 (12)	20 (9)
Ginkgo biloba	12 (5)	11 (5)
St. John's Wort	7 (3)	5 (2)
Nutritional Supplements		
Ensure	16 (7)	14 (6)
Fish oils	9 (4)	8 (4)
Miscellaneous		
Shark cartilage	2 (1)	2 (1)
Melatonin	5 (2)	5 (2)
Evening Primrose	6 (3)	5 (2)
Glucosamine/ Chondroitine	6 (3)	6 (3)
CoQ 10	6 (3)	5 (2)

[a] Values in parenthesis are percent of total respondents (N=220).

Table 3. Multivariate logistic regression results for factors associated with any antioxidant supplement use and use solely after diagnosis with breast cancer.

Characteristic	Any Use			New use after diagnosis		
	%	OR*	95% CI*	%	OR*	95% CI*
Age						
< 60	69	1.00		35	1.00	
60-69	58	1.12	0.5-2.3	26	0.88	0.4-1.9
70+	65	1.99	0.7-5.4	24	0.78	0.3-2.3
BMI						
<25	65	1.00		33	1.00	
25+	62	1.05	0.6-2.0	23	0.73	0.4-1.5
SES						
Low	47	1.00		23	1.00	
Medium	58	1.93	0.8-4.9	21	0.74	0.3-2.2
High	78	3.80	1.4-10.2	42	1.58	0.6-4.5
Stage						
I	64	1.00		31	1.00	
II or IIIB	62	0.85	0.4-1.7	26	0.67	0.3-1.4
History of Use of OC						
No	60	1.00		26	1.00	
Yes	77	2.26	0.9-5.6	42	1.69	0.8-3.8
Tamoxifen						
No	60	1.00		29	1.00	
Yes	70	1.99	1.0-3.9	29	0.99	0.5-1.9
Caloric intake/ day						
< 1700	60	1.00		31	1.00	
> 1700	69	1.60	0.8-3.1	27	0.75	0.4-1.5
Exercise						
None	57	1.00		15	1.00	
Past	58	0.87	0.4-2.0	29	1.82	0.7-4.8
Current	71	1.55	0.7-3.2	39	2.62	1.2-5.9
Smoking status						
Never	65	1.00		25	1.00	
Past	63	0.73	0.4-1.5	38	1.67	0.8-3.4
Current	60	0.56	0.2-1.5	24	0.87	0.3-2.6
Medicinal herbs						
Non-user	57	1.00		24	1.00	
User	82	3.72	0.6-8.9	45	1.84	0.9-3.8

OR: Odds Ratio, CI: Confidence Interval

Antioxidant supplements and risk of breast cancer recurrence and breast cancer related mortality among postmenopausal women.

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Support: This work is supported by a pre-doctoral traineeship award from U.S. Department of Defense Breast Cancer Research Program (DAMD-17-01-1-0350).

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Abstract

Despite widespread use, only a few clinical or epidemiological studies have examined the relationship between antioxidant supplements and risk of breast cancer recurrence or breast cancer-related mortality. We used proportional hazards and logistic regression modeling to estimate rate ratios and odds ratios for recurrence and mortality among 385 postmenopausal women diagnosed with breast cancer between 1986 and 1988 enrolled into a case-control study on diet and cancer. Women were re-contacted to ascertain the use of nutritional supplements during 12 to 14 years of follow-up time. In multivariable models, antioxidant supplement users compared with non-users were less likely to have a breast cancer recurrence or breast cancer related death (OR=0.54; 95% CI, 0.27-1.04). Vitamin E supplements showed a modest protective effect when used for more than 3 years (OR=0.33; 95% CI, 0.10-1.07). Pre-morbid dietary intake of vitamins C or E from diet and/or supplements showed no relationship with risk. Risks of recurrence and disease related mortality were reduced among women using antioxidant supplements and vitamin E supplements for more than 3 years. Recall bias among proxy respondents for women who had died during follow-up may have contributed to these findings. This study provides limited support for the hypothesis that antioxidant supplements may reduce the risk of breast cancer recurrence or breast cancer related mortality.

Introduction

Antioxidants have been shown to reduce the risk of cancer in laboratory and epidemiological studies, possibly through their ability to neutralize free radical damage (1). In breast cancer, vitamin C and E supplements may also induce mammary cell differentiation (2), apoptosis (3, 4), and may inhibit tumor progression (5). Several epidemiological studies have shown beneficial effects of vitamins C and E from diet and/ or supplements in breast cancer prevention (6-9), whereas others have reported no relationship (10,11). Dietary intakes of vitamins C and E from foods were protective in three case-control studies of breast cancer mortality (12-14), but they were found not to be related to risk in a case-control (15) and in one large cohort study (16). In two small clinical trials, where breast cancer patients were supplemented with antioxidants, one showed a slight decrease in mortality (17) and the other reported no affect on prognosis (18).

With improvements in early detection and treatment of breast cancer, survival rates are increasing (19). Yet the most imminent fear for women successfully treated for their primary breast cancer is a recurrence, where risk of five-year relapse ranges between 10 - 20% (20-21) depending on stage at diagnosis. Predictors of breast cancer recurrence and disease-related mortality have primarily focused on tumor-related characteristics, in addition to clinical treatments. Several reports indicate that cancer stage, histological features (mitotic index nuclear grade, and combined histological grade), lymph node positive and ER negative status are associated with increased risk of breast cancer recurrence and cancer related mortality (12,15,23). But the majority of women who survive breast cancer attribute this to diet and lifestyle changes after diagnosis (22).

This analysis focuses primarily on antioxidant intake from individual supplements. We have also examined antioxidants from diet, multivitamins, or from all sources combined. Most single-source antioxidant supplements contain doses well in excess of those typically consumed from diet, provided by multivitamin sources, or is recommended for daily consumption (e.g., U.S. RDA). Given the evidence that women with breast cancer use vitamin supplements in amounts and in frequencies greater than the general population (24-25), ascertaining the effectiveness of these supplements in preventing breast cancer recurrence or increasing disease-free survival is an important issue relating to this common disease.

Methods

Study population:

The methods for initial recruitment of the women enrolled into this study have been described in detail elsewhere (26-27). In brief, participants with a diagnosis of invasive breast cancer were recruited from five Boston area hospitals between 1986 and 1988 and a total of 407 postmenopausal breast cancer cases were enrolled into a population-based case-control study (26). Cases were post-menopausal at diagnosis, with no history of prior cancer other than non-melanoma skin cancer. Women with early stage breast cancer (AJCC stage I, II, III) without distant metastases were recruited. Subsequent to diagnosis, 27% of this population was found to have positive lymph nodes, though no distant metastases were identified. Follow-up on cases was conducted as part of the FASTCAB study (Fatty Acid

Stores Tumor Characteristics and Breast Cancer) where vital status, tumor block and medical record information have been collected.

Follow-up questionnaires were mailed in November 1999 to the 393 women from the FASTCAB population who had not refused further participation, and subsequent mailings were sent to non-respondents in January 2000. Next of kin (NOK) of cases who had died (N=123) during the follow-up period (1989 – 1999) received a version of the questionnaire modified for proxy respondents.

The response rate was 65% for living cases and 38% for proxy respondents of cases who had died during the follow-up period. Of 270 living cases, 22 (8.1%) did not receive questionnaires due to inability to trace their addresses through credit and other computer-based searches and were considered lost to follow-up, 10 (2.7%) refused participation and 64 (23.7%) were non-respondents after three consecutive mailings. Of the 122 NOK (proxies) identified, 23 (18.9%) did not receive questionnaires due to inability to trace their addresses through credit and other computer-based searches and were considered lost to follow-up, 11 (9%) refused participation and 42 (34.4%) were non-respondents.

Breast Cancer Recurrence and Survival (Outcome): Death data was retrieved from the National Death Index (NDI) and Social Security Death Index (SSDI) searches followed by requests for relevant death certificates from state vital records offices. Information on breast cancer recurrence was obtained from the medical records and crosschecked with respondent self-report on the follow-up questionnaire. Cause of death was determined through death certificates, which were coded by a trained Nosologist, deaths with a cause, or a contributing cause of breast cancer were considered breast cancer related mortality events. Breast cancer recurrences were determined from review of the cases' medical records with additional verification from follow-up questionnaires. Nonfatal breast cancer recurrences were combined with breast cancer related mortality events to provide the single outcome variable used in analysis for this report.

Supplement assessment: The questionnaire was a self-administered survey and took approximately 15 minutes to complete in focus group testing. For living cases and NOK (proxies) of the cases who had died, forms of the questionnaire differed in the style of question wording and the omission of current age and weight in the NOK version.

The questionnaire consisted of 10 pre-coded closed response questions, a list of supplements (three categories: herbals, vitamins and minerals, and nutritional supplements) and 10 lines to insert the name of the supplement(s) selected from the list and answer questions regarding each supplement selected. The pre-coded questions concern the use of hormone replacement therapy (HRT), Tamoxifen, alcohol, smoking and consumption of soy containing foods. Classifications included never, past, and current use. For past and current use, we also assessed the length of time of usage with approximate stop or start dates, respectively. Two questions were designed to verify breast cancer recurrence data obtained from medical records.

Whether the supplement was used before, after, or both before and after diagnosis of breast cancer, the total length of time of use in years, frequency per month and the approximate or average dose using the appropriate units (e.g., mg or IU) was assessed. "Any use" of supplements was defined by supplement use reported as before diagnosis, only after

diagnosis or both before and after diagnosis. Use of supplements only after diagnosis was considered "new use".

Supplements were categorized in accordance with previous literature (24). Antioxidants were defined as containing primarily antioxidant nutrients, including one or more of the following: β-carotene, vitamin C, vitamin E and selenium. Multivitamins are formulations containing a variety of vitamin and minerals, primarily one or more vitamins in combination with one or more mineral. A 124-item Willett food frequency questionnaire (FFQ) was used as part of the original case control study (1986-1988). Supplement and dietary assessment from the FFQ concerned the year prior to diagnosis of breast cancer. From this FFQ, vitamin C, E, beta-carotene, selenium and calcium supplement use as well as total intake of vitamin C and E from foods was obtained, and used to assess pre-diagnostic intake.

Statistical analysis:

Descriptive statistics were employed to compare factors by respondent status to the follow-up questionnaire. Differences between respondents and non-respondents were estimated by using Chi square statistics for categorical variables. Proportional hazards models were used to examine the independent associations between pre-morbid antioxidant nutrients from food and/ or supplements and breast cancer recurrence and disease related mortality, as well as antioxidant supplements consumed after diagnosis. This approach allows for the varying lengths of time to recurrence or death and for control of other variables. The Cox model estimates rate ratios, which allow for approximations of relative risk estimates. Person time was calculated from date of study entry (1986-1988) and ended with either a recurrence date, death date or date the questionnaire was returned. Since a single outcome was used, which included either a recurrence or a death event, women who had both a recurrence and breast cancer related death, time was calculated to the first outcome, recurrence.

Logistic regression was used to examine antioxidant supplement use collected from the follow-up questionnaire. The five-year logistic regression analysis was performed since supplement use obtained from the follow-up questionnaire subsequent to breast cancer recurrence and mortality events represents time-dependent covariates. As a result, a logistic model was used to analyze lifetime, post-diagnosis and duration of supplement use in the post-diagnosis period, as opposed to pre-morbid diet, which allowed for a proportional hazards analysis. Cases were excluded for deaths within five-years of diagnosis to allow all cases an opportunity for post-diagnosis supplement intake.

As an exploratory step, a step-wise model was used to determine which variables were most strongly related to the breast cancer outcomes at the significance level of $P<0.20$. All Cox and logistic regression models were adjusted for age at diagnosis and other potential risk factors. These factors included: Tumor stage (I, II-IIIB) age at menopause, age at first pregnancy, parity (no children, ≥ 1), hormone replacement therapy, radiation therapy, chemotherapy, Tamoxifen, exercise (non-exercise, past, current), smoking (non-smokers, past, current), BMI (<25.0, ≥25.0), use of oral contraceptives, and use of other dietary supplements (multivitamins, calcium and medicinal herbs).

The only confounders to have an appreciable effect on our results were tumor stage, radiation therapy, Tamoxifen use, and exercise, which were based on the best-fit stepwise model. All models included dummy variables for antioxidant (at least one of the following supplements: C, E, beta-carotene and/ or selenium), vitamin C or E supplements. Models

analyzed these supplements as dichotomous (users, non-users) and by duration of use (non-users, <3 years, ≥3 years). Cut-points for duration of use were determined by the median value among users in the non-outcome population with non-use as the reference group. Vitamin E from food and supplement sources pre-diagnosis was determined by the Willett FFQ and categorized by the median or thirds in the non-outcome cohort.

All models were rerun and stratified by whether the respondent was a living case or a proxy in order to assess any possible influence of measurement error on behalf of the NOK. To determine whether the association of vitamin E supplement use was modified by radiation therapy, as hypothesized elsewhere (28), we examined stratified results and used an interaction term. The interaction term multiplied a dichotomous vitamin E variable (ever, never) by whether radiation therapy was received (yes, no). We did not stratify results by dietary intake of vitamin C or E, since this information would have come from the baseline FFQ at study entry and may therefore misrepresent intake throughout the post-diagnosis period. Cut-points for total consumption of pre-morbid vitamin C and E from food sources and from food and supplement sources combined were determined by using tertiles in the control (disease-free survivors) population.

Results

After follow-up attempts on 385 women, 220 responded to the follow-up questionnaire. They tended to be younger than non-respondents. The mean age at diagnosis was 62.1 (SD=7.8, range=47-93) among respondents and 66.3 (SD=8.7, range=47-89) among non-respondents (Table 1). Respondents were more likely to have been diagnosed with a stage I cancer (<2 cm) than were non-respondents (68% and 59%, respectively). Among respondents, 58 (26%) cases of breast cancer recurrence or breast cancer related deaths were identified during 12-14 years of follow-up time. Specifically, 41 (18%) recurrences were identified and 23 (10.5%) deaths were attributed to breast cancer. Rates of breast cancer recurrences and breast cancer deaths among non-respondents (27%) were comparable to those for respondents.

Overall, lifetime (ever) antioxidant vitamin supplement use was associated with a lower risk of breast cancer recurrence or mortality; see Table 2. Ever use of vitamin C and E supplements demonstrated moderate protective effects. For vitamin E supplement use, nearly a halving of risk was observed (OR=0.55; 95% CI, 0.28-1.08). When analyses were restricted to living cases, the results were attenuated (OR=0.65; 95% CI, 0.28-1.49). When women who used either a vitamin C or vitamin E supplement were combined in a single group, a reduced risk of breast cancer recurrence and mortality was also observed (OR=0.56; 95% CI, 0.28-1.11). Again, this estimate was attenuated when proxy questionnaires were removed (OR=0.79; 95% CI, 0.32-1.96). Results, when restricted to the small subset of proxy respondents were similar to the results observed for living cases in all analyses. Consistency of results less than 1.0 were observed for all analyses and sub-analyses of lifetime supplement use.

Use of Multivitamins was, however, not related to disease-free survival (OR=0.91; 95% CI, 0.52-1.59). Nearly one-third (29%) of antioxidant supplement users were not multivitamin consumers, suggesting that the effects and users of antioxidant supplements differed from that of multivitamins.

To estimate the effects of long-term duration of use, we evaluated two categories for length of use among vitamin C and E users. The multivariate-adjusted ORs for breast cancer recurrence or breast cancer mortality associated with increasing years of supplement use are presented in Table 3. Among the total cohort vitamin E was associated with a non-significant reduced risk when consumed for less then three years (OR=0.50; 95% CI, 0.17-1.48), and a moderate to strong reduction in risk when taken for greater than three years (OR=0.33; 95% CI, 0.13-1.07). A similar trend was observed for vitamin C. Consumption of either vitamin C or vitamin E for four or more years showed a strong and significant reduction in risk among the total cohort (OR=0.30; 95% CI, 0.11-0.76), and a modest effect among living cases (OR=0.50; 95% CI, 0.18-1.40). When analyses were restricted to living cases, as expected, attenuation of the ORs was observed, particularly in the less than three years category for vitamin C. We were not able to perform analyses on the subset of proxy cases due to limits in the number of respondents.

When consumed solely after diagnosis, vitamin E supplementation again appeared to be associated with a slight reduced risk of recurrence and mortality (OR=0.75, 95% CI, 0.34-1.76) in the total cohort. Vitamin C, however, did not show a protective relationship when consumed strictly post-diagnosis (OR=0.90, 95% CI, 0.35-2.23).

The multivariable adjusted ORs when stratified by living and proxy respondents for total antioxidants, vitamins C and E supplements showed little difference. For example, the adjusted OR for vitamin C use was 0.64 (95% CI, 0.32-1.27) in the total cohort, and ORs of 0.77 (95% CI, 0.34-1.84) and 0.75 (95% CI, 0.16-3.52) were computed among living cases and proxy respondents, respectively. The crude OR estimates for vitamin C, as shown in table 2b, were slightly attenuated at 0.86 (95% CI, 0.47-1.58) in the total cohort, with stratified results of 1.22 (95% CI, 0.58-2.57) and 0.59 (95% CI, 0.17-2.04) among living cases and proxies, respectively.

The adjustment for covariates had a profound effect on the stratified ORs. In order to explain the effects observed for lifetime and post-diagnosis supplement use, an examination of the crude results and the distribution of covariates stratified by response status were performed. As demonstrated in Table 2c, the association between covariates included in the final logistic model and vitamin C supplement use differed by response status. Use of vitamin C supplements was significantly more common among living cases (OR=3.33; 95% CI, 1.74-6.39) who received Tamoxifen therapy. However, and opposing effect was observed among proxy respondents, where the odds of taking vitamin C supplements were less among women receiving Tamoxifen therapy (OR=0.67; 95% CI, 0.19-2.31). A similar trend was observed for disease stage, where stage I tumors were slightly more common among proxy respondents who used vitamin C supplements. And past exercise was less likely to be associated with vitamin C supplement use among proxy cases.

The adjusted ORs for each of these covariates, as seen in Table 2d, demonstrated effect modification for tumor stage. Tumor stage was associated with an increased risk of recurrence and mortality among proxies and exerted no effect among living cases on recurrence. Both past exercise and Tamoxifen were associated with an increased risk of recurrence and/or mortality among living cases and proxy cases, though the magnitude of effect differed somewhat.

Pre-morbid vitamin E from foods showed little to no relationship for consumption of 7.75 mcg/day or greater among non-users of vitamin E supplements (RR=0.74, 95% CI=0.28-1.99) who responded to the follow-up questionnaire. No association with greater

than 120mg of vitamin C from foods was observed. Examining vitamin E supplement intake prior to diagnosis of breast cancer showed little to no relationship with risk (RR=0.85, 95% CI, 0.40-1.82) for greater than 40 mg/day compared with non-use or use less than 40 mg/day among respondents to the follow-up questionnaire. Consumption of 500 mg/day of vitamin C supplements was unrelated to risk (RR=1.15; 95% CI, 0.58-2.30). Pre-diagnostic vitamin E from food sources and supplements was not associated with risk. The relative risk estimate for greater than 35 mg/day of vitamin E from foods and supplements assessed in the pre-diagnosis period was 0.99 (0.62-1.59) compared with less than 8 mg/day. Likewise consumption of greater than 500 mg/day of vitamin C from foods and supplements was not associated with risk (RR= 1.13, 95% CI, 0.71-2.34).

In the total cohort (N=385), including non-respondents who had completed the Willett-FFQ (N=345), pre-morbid vitamin E and C from foods and supplements may be related to an increased risk of breast cancer recurrence and mortality, as can be seen in Table 4. Strong correlation was observed between vitamin E and C consumption from foods and/ or supplements (R^2=0.55, p<0.001), in which risk estimates were similar, and around unity, for each of these nutrients. Vitamin E from foods and supplements was not associated with risk for the highest (>35mg/day) levels of consumption RR=1.14 (95% CI, 0.59-2.22). Vitamin C from foods and supplements combined did however show an increased risk for the highest level of consumption (>500mg/day) RR=1.56 (95% CI, 0.95-2.75), though not statistically significant.

Whereas this study was limited in sample size and did not have strong statistical power, we tested whether the ORs for vitamin E from supplements consumed after diagnosis differed by radiation therapy. Women who did not receive radiation therapy appeared to derive benefit from vitamin E supplementation (OR=0.49; 95% CI, 0.25-0.98) whereas no such relationship was observed among women who did have radiation; see Table 5. The independent effect of radiation therapy also showed benefit, particularly among living cases. The modification of the ORs was observed among living cases only. The interaction for living cases, assessed on the multiplicative scale, was not significant for this sample size (P=0.10). However, the OR for doubly exposed group (women who received radiation and took vitamin E supplements) would have been predicted to have been less than the protective independent effects of either radiation or vitamin E. Here the OR for the doubly exposed group was greater. Among proxy respondents, the very small sample sizes precluded multivariable analyses. Crude results showed less of a benefit for vitamin E, independent of and in addition to radiation therapy.

Discussion

An urgent question raised among breast cancer survivors is whether antioxidants are beneficial, detrimental or of no use to women diagnosed with breast cancer. Among this small cohort of postmenopausal women, no evidence for a detrimental effect of vitamin C or E supplement use was found and limited support for the hypothesis that antioxidant supplement use was associated with a decreased risk of breast cancer recurrence and breast cancer-related mortality. Higher pre-morbid consumption of vitamin C and E from diet was unrelated with breast cancer prognosis in our study, contrasting with prior reports showing benefit for higher vitamin C consumption. For higher pre-morbid vitamin E intake however, our results were consistent with no association observed.

Few studies have addressed survival – and these few have done so generally either with small cohorts or having assessed only pre-diagnosis antioxidant intake and often omitting supplement use. Several studies that have examined the relationship of dietary antioxidants with breast cancer prognosis found an association with higher vitamin C intake (12-14, 29-30). Others (15,16) failed to detect any relationship with either vitamin C or vitamin E intake. A study of 451 breast cancer cases in Australia found a slight decline in risk of death for the highest level of pre-morbid consumption of vitamin C from diet, but no dose-response relationship (14). Jain et al. (13) reported a significantly decreased risk of death for higher pre-morbid dietary intakes of vitamin C among 1270 breast cancer cases. Hebert reported approximately a 40% reduced risk of breast cancer recurrence or mortality for each 100 mg change in vitamin C intake from before to after diagnosis in a study of 472 women diagnosed with early stage breast cancer (12).

Consistent with our finding for pre-morbid dietary intake of vitamins C and E, data from the Iowa Women's Health Study cohort (16), found antioxidant micronutrients from diet and supplements to be unrelated to breast cancer survival. Likewise, Saxe and colleagues found pre-morbid intake of vitamin C not to be associated with either a breast cancer recurrence or disease-free survival (15). Both of these reports observed few recurrences and deaths.

This study presented limited evidence for a dose-response relationship between duration of use of vitamin C or E supplements and risk of recurrence and mortality. Long term use of these supplements, particularly vitamin E, appeared to be related to a modestly reduced risk of recurrence and mortality. Duration of use was not precluded to the post-diagnosis period. In fact, these supplements were consumed either pre-diagnosis, post-diagnosis or during both periods. However, most vitamin supplement use occurred after diagnosis of breast cancer (27), where over 75% of reported vitamin C and E supplement intakes were after diagnosis, whether this was continued from pre-diagnosis or new use. No previous reports have analyzed duration of vitamin C or E supplements with breast cancer prognosis. Our results, particularly those that were restricted to living cases, were consistent with a study of antioxidant supplements and breast cancer incidence. Results from the Carolina Breast Cancer Study (5) provided suggestive evidence that duration of vitamin C and E supplement use may be related to a reduced risk of breast cancer. An odds ratio of 0.63 (95% CI, 0.34-1.20) was reported among white women who consumed vitamin E supplements for greater than three years. No dose-response relationship was observed between duration of use and risk of breast cancer.

Neither in our cohort nor in other cohorts was multivitamins related to risk, regardless of length of years consumed (31). One possible explanation is that antioxidant concentrations in multivitamins are significantly lower that those in individual vitamin supplements, and a beneficial threshold effect may not have been met with multivitamins.

Pre-morbid intake of vitamins C and E from dietary sources, independent of supplements, was found not to be related to risk of breast cancer recurrence or survival, although a very slight benefit was observed for the highest tertile of vitamin E intake among non-users of vitamin E supplements. Highest pre-morbid levels of vitamin C or E from food combined with supplements showed no association with risk. These results do not support the hypothesis that consumption of antioxidants from dietary sources and/ or supplements prior to diagnosis of breast cancer were associated with subsequent breast cancer prognosis.

Based on prior reports (28,32) we tested the whether or not an interaction between vitamin E intake and radiation therapy existed. The hypothesized interaction was that

vitamin E, independent of radiation would be beneficial, but vitamin E supplementation among women who received radiation therapy would exert an even stronger protective association with recurrence and death. A synergistic relationship, marked by enhanced treatment efficacy with antioxidant co-administration, has been described previously, but not tested in humans (32).

A protective effect for vitamin E supplements was observed among women who did not receive radiation therapy. Among women who received radiation therapy and reported use of vitamin E supplements after diagnosis, no benefit was observed. Whereas there was no statistically significant evidence for an interaction between vitamin E supplements and radiation therapy, the p-value of 0.10, given the limited sample size, suggested the presence of an interaction. A larger sample size may have yielded evidence for a significant interaction. This study had limited statistical power, and detection of significant interactions between antioxidants and other factors would have required a larger degree of effect modification. The result is interesting nonetheless, and lends credence for future research to specifically examine the use of vitamin supplements during radiation therapy. A major limitation for this analysis was the inability to determine if in fact supplement use in the post-diagnosis period actually occurred during the radiation therapy or afterwards.

Whereas slight protective effects were observed, particularly for long-term use of vitamins C or E, limitations may have influenced the results. Some caveats that may need to be considered are the removal of proxy questionnaires, recall bias, residual confounding and alternative hypotheses. When analyses excluded proxy questionnaires, the observed reduced risks were slightly attenuated towards no association. However, results for proxy respondents only, while a very limited sample size, were also similar to those of living cases. The fact that there were little differences between these groups was unexpected, because the crude ORs demonstrated effect modification by respondent status. The crude ORs for proxy respondents were suggestive for strong protective associations. However, these results were dramatically changed upwardly towards no association after adjustment for confounders. This was certainly a result of differences in the distribution of covariates between living and proxy cases. As demonstrated, the coefficients for the covariates included in the models differed by response status. One example was the interaction observed for Tamoxifen therapy, in which effect modification was apparent between living cases and proxy respondents.

More than half of the proxy respondents were spouses, while the remainders were sons, daughters or siblings. Moderate to strong agreement for proxy questionnaire responses has been reported particularly when the proxy was spouse (33). Recall bias may have been influential in the classification of ever versus never supplement use and duration of use. Proxy respondents may have been unable to accurately recount past usage of supplements, exercise or the use of Tamoxifen for the person they represented.

Exclusion of proxy questionnaires also changed the outcome assessed, by removing mortality events and leaving non-fatal breast cancer recurrences. Among proxy respondents, there were 12 breast cancer related deaths, whereas 35 of the remaining deaths were a result of other causes. Since the survey focuses on past and current use of supplements, cases may have to recall supplement use as much as 10-12 years earlier. While the proxy respondent group naturally included all of the mortality events, it included a similar proportion of breast cancer recurrence as living cases. The removal of this group also significantly reduced power

of the study to detect significant associations. The potential for bias, therefore, cannot be separated from an actual effect in this study.

Alternative hypotheses may explain these findings if, for example, vitamin supplementation is a marker for a healthier lifestyle. Previous studies (5,24,34) have shown that women with breast cancer who consume vitamin supplements tend to be of higher socioeconomic status, with lower BMI, more likely to engage in regular physical exercise, and to eat higher concentrations of fruits and vegetables in their diets. These factors are typically associated with reduced risks of chronic disease and improved disease prognosis. Although many of these factors were controlled for in our multivariable models, confounding by other factors associated with these variables and not assessed in our study may have influenced the observed modest reductions in risk.

One of the strengths of this study was the use of measures of supplement use both before and after diagnosis of breast cancer, particularly since long-term use is more validly assessed with the use of multiple questionnaires (35). While pre-diagnosis consumption of vitamin E showed little to no effect on risk, the continuation of supplement use or new use in the post-diagnosis period slightly reduced the risk of recurrence and breast cancer related mortality. Diet has been shown to change considerably after diagnosis of breast cancer (34). Previous work showed that a significant increase in vitamin supplement use occurs after diagnosis of breast cancer. Intake prior to diagnosis of breast cancer was analyzed by increasing dose, and concerned the year prior to diagnosis. Information regarding years of supplement use from the baseline FFQ was not available. Perhaps length of use of vitamin C or E supplements pre-diagnosis would have proven more relevant than the dose utilized. The longer the use of vitamin E, the greater the benefit received appeared to be. Use begun before breast cancer and continuing after diagnosis may have elicited the modest reduction in risk observed in this cohort, under the assumption that the longest duration of intake most likely consisted of pre and post diagnosis use. When post-diagnosis only use was analyzed, the results showed slightly less apparent benefit.

The findings from this study lend limited evidence for the hypothesis that antioxidant supplements reduce the risk of breast cancer recurrence and breast cancer related mortality. Pre-morbid dietary intake of antioxidant supplements did not appear to be related to subsequent risk of recurrence or death, nor were pre-morbid vitamin C and E from both diet and supplements combined. Increasing lengths of use of vitamins C or E supplements appeared to be associated with a modest reduction in risk, but it is unclear what influence bias and error may have played with these finding. Nonetheless, vitamins C and E from diet or supplements have not shown a detrimental effect on breast cancer prognosis, and lifetime use of vitamins C and E supplements does appear offer modest benefit. More research is needed to establish whether use of antioxidant supplements after diagnosis confers clear-cut prognostic benefits for women with diagnosed breast cancer.

Acknowledgements:

The authors would like to thank Drs Robert Millikan, John Anderson and Mike Symons for their careful review of this manuscript.

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Table 1. Demographic characteristics at time of diagnosis or study entry of postmenopausal breast cancer cases and by response to follow-up questionnaire.

Characteristic (%)	Respondents N=220	Non-respondents N=165	p-value
Age			
Mean (SD)	62.1 (7.8)	66.3 (8.7)	0.001
Deaths[1]	21.4	41.7	0.001
Stage I	68.2	58.6	0.05
Node negative	81.8	80.3	0.69
Radiation therapy	28.7	20.8	0.07
Chemotherapy	6.4	5.9	0.83
Tamoxifen	38.2	NA[3]	NA[3]
Vitamin C[2]			
60-500 mg/day	9.4	13.8	
>500 mg/day	17.2	17.6	0.41
Vitamin E[2]			
10-40 mg/day	12.0	12.6	
>40 mg/day	16.7	17.6	0.56

[1]1986-1988 FFQ used to assess pre-diagnosis
[2]Pre-morbid supplement use.
[3]NA=not available.

Table 2a. Use of antioxidant supplements after diagnosis and risk of breast cancer recurrence or breast cancer related mortality among women diagnosed with breast cancer.

Vitamin Supplement(s)	$(N_0)^1$ N=162	$(N_1)^1$ N=58	Total Cohort (N=220) OR (95% CI)[2]	Living Cases (N=173) OR (95% CI)[2]	Proxy Cases (N=47) OR (95% CI)[2]
Antioxidants[3]					
Non-users	58	26	1.0	1.0	1.0
Ever	104	32	0.54 (0.27-1.04)	0.76 (0.31-1.88)	0.60 (0.16-2.46)
Vitamin E					
Non-users	78	33	1.0	1.0	1.0
Ever	84	25	0.55 (0.28-1.08)	0.65 (0.28-1.49)	0.77 (0.19-3.27)
Vitamin C					
Non-users	89	34	1.0	1.0	1.0
Ever	73	24	0.64 (0.32-1.27)	0.77 (0.34-1.84)	0.75 (0.16-3.52)
Vitamin C or E					
Non-users	60	26	1.0	1.0	1.0
Ever	102	32	0.56 (0.28-1.11)	0.79 (0.32-1.96)	0.61 (0.17-2.45)

[1] N_0=Disease-free survivors, N_1=Women with recurrences and/ or disease related mortality.
[2] Multivariate logistic regression models adjusted for age at diagnosis; age at menopause; tumor stage; Tamoxifen, radiation therapy use; hormone replacement therapy; smoking (past, current); Exercise (past, current) and dietary intake of vitamin C or E.
[3] Antioxidant supplements are the use of at least one individual vitamin C, E, beta-carotene, selenium or antioxidant combination supplement.

Table 2b. Use of antioxidant supplements after diagnosis and risk (crude estimates) of breast cancer recurrence or breast cancer related mortality among women diagnosed with breast cancer.

Vitamin Supplement	Total (N=220) OR (95% CI)	(N_0)[1] N=138	(N_1)[1] N=35	Living Cases (N=173) OR (95% CI)	(N_0)[1] N=24	(N_1)[1] N=23	Proxy Cases (N=47) OR (95% CI)
Antioxidants[3]							
Non-users	1.0	48	11	1.0	10	15	1.0
Ever	0.68 (0.37-1.26)	90	24	1.16 (0.53-2.57)	14	8	0.38 (0.12-1.24)
Vitamin E							
Non-users	1.0	60	16	1.0	18	17	1.0
Ever	0.70 (0.38-1.28)	78	19	0.91 (0.43-1.92)	6	6	0.44 (0.13-1.18)
Vitamin C							
Non-users	1.0	74	17	1.0	15	17	1.0
Ever	0.86 (0.47-1.58)	64	18	1.22 (0.58-2.57)	9	6	0.59 (0.17-2.04)

[1] N_0=Disease-free survivors, N_1=Women with recurrences and/ or breast cancer related

Table 2c. The odds of vitamin C supplement use across covariates and stratified by response status.

Covariate	$(C_0)^1$ N=123	$(C_1)^1$ N=97	Total Cohort (N=220) OR (95% CI)	Living Cases (N=173) OR (95% CI)	Proxy Cases (N=47) OR (95% CI)
Exercise					
None	43	29	1.0	1.0	1.0
Past	29	19	0.79 (0.41-1.51)	0.98 (0.46-2.05)	0.48 (0.11-2.05)
Current	51	49	1.44 (0.84-2.46)	1.16 (0.64-2.13)	NA
Tamoxifen					
None	86	50	1.0	1.0	1.0
Ever	37	47	2.18 (1.25-3.08)	3.33 (1.74-6.39)	0.67 (0.19-2.31)
Tumor Stage					
I	82	65	1.0	1.0	1.0
≥II	39	29	0.93 (0.53-1.67)	0.89 (0.45-1.70)	1.43 (0.40-5.17)

[1] C_0=Vitamin C supplement non-users, C_1=Vitamin C supplement users.

Table 2d. The adjusted risk estimates for select covariates and risk of breast cancer recurrence or breast cancer related mortality among women diagnosed with breast cancer.

Vitamin Supplement(s)	$(N_0)^1$ N=162	$(N_1)^1$ N=58	Total Cohort (N=220) OR (95% CI)[2]	Living Cases (N=173) OR (95% CI)[2]	Proxy Cases (N=47) OR (95% CI)[2]
Exercise					
None	56	16	1.0	1.0	1.0
Past	29	19	2.07 (0.89-4.79)	3.89 (0.93-16.32)	1.95 (0.43-8.95)
Current	77	23	0.97 (0.45-2.09)	3.22 (0.88-11.70)	NA
Tamoxifen					
None	113	23	1.0	1.0	1.0
Ever	49	35	3.65 (1.91-6.95)	3.27 (1.47-7.22)	7.38 (1.63-33.40)
Tumor Stage					
I	112	35	1.0	1.0	1.0
≥II	47	21	1.38 (0.70-2.71)	0.82 (0.34-1.99)	5.32 (1.06-26.60)

[1] N_0=Disease-free survivors, N_1=Women with recurrences and/ or disease related mortality.
[2] Multivariate logistic regression models adjusted for tumor stage; Tamoxifen, and Exercise (past, current).

Table 3. Length of time in years of vitamin E and C use and risk of breast cancer recurrence and breast cancer related mortality among postmenopausal women.

Vitamin Supplement	$(N_0)^1$ N=162	$(N_1)^1$ N=58	Total Cohort (N=220) OR (95% CI)[2]	Living Cases (N=173) OR (95% CI)[2]
Vitamin E				
Non-use	88	39	1.0	1.0
0-2 years	33	12	0.50 (0.17-1.48)	0.81 (0.26-2.58)
≥3 years	41	7	0.33 (0.10-1.07)	0.52 (0.15-1.83)
Vitamin C				
Non-use	108	42	1.0	1.0
0-3 years	25	10	0.61 (0.24-1.57)	0.98 (0.35-2.72)
≥4 years	29	6	0.34 (0.11-0.97)	0.59 (0.19-1.84)
Vitamin C or E				
Non-use	79	35	1.0	1.0
0-3 years	37	14	0.61 (0.27-1.39)	0.77 (0.30-1.97)
>4 years	43	7	0.30 (0.11-0.76)	0.50 (0.18-1.40)

[1] N_0=Disease-free survivors, N_1=Women with recurrences and/ or breast cancer related mortality.

[2] Multivariate logistic regression models adjusted for age at diagnosis; age at menopause; tumor stage; Tamoxifen, radiation therapy use; hormone replacement therapy; smoking (past, current); Exercise (past, current) and dietary intake of vitamin C or E.

Table 4. Pre-morbid intake of Vitamin E and C from foods and/ or supplements among the total cohort of breast cancer cases who completed the Willett FFQ (N=345) and risk of breast cancer recurrence and breast-cancer related mortality.

Nutrient/ Source	N_0	N_1	Cut-points	RR (95% CI)
Vitamin E	184	64	None	1.0
Supplements	28	15	<30	1.57 (0.89-2.79)
	40	20	>30	1.24 (0.72-2.13)
Vitamin E	82	36	<6.5	1.0
Foods	88	28	6.5-9.15	0.83 (0.42-1.65)
	80	33	>9.15	1.27 (0.67-2.42)
Vitamin E	102	39	<8	1.0
Foods and	102	33	8-35	0.96 (0.52-1.76)
supplements	48	27	35+	1.14 (0.59-2.22)
Vitamin C	186	64	None	1.0
Supplements	29	11	<500	1.22 (0.89-2.79)
	87	24	>500	1.60 (0.72-2.13)
Vitamin C	83	33	<121.5	1.0
Foods	88	34	121.6-180	0.96 (0.49-1.89)
	80	32	>180	1.21 (0.48-3.04)
Vitamin C	94	30	<150	1.0
Foods and	113	43	150-500	1.16 (0.75-1.78)
supplements	45	26	>500	1.56 (0.95-2.75)

[1]N_0=Disease-free survivors, N_1=Women with recurrences and/ or breast cancer related mortality.
[2]Multivariable proportional hazards models adjusted for age at diagnosis; age at menopause; tumor stage; Tamoxifen, radiation therapy use; hormone replacement therapy; smoking (past, current); Exercise (past, current) and dietary intake of vitamin C or E.

Table 5. Odds ratios for breast cancer recurrence or breast cancer related mortality among women diagnosed with breast cancer stratified by radiation therapy who used vitamin E supplements after diagnosis.

Vitamin E Supplement	Total Cohort[1] (N=220)				Living Cases Only[1] (N=173)				Proxy Cases Only[2] (N=47)			
	Radiation Therapy				Radiation Therapy				Radiation Therapy			
	Yes		No		Yes		No		Yes		No	
	N_0	N_1	N_0	N_1	N_0	N_1	N_0	N_1	N_0	N_1	N_0	N_1
Non-use	20	9	58	25	17	3	43	13	3	6	15	11
Ever	24	10	60	15	24	8	54	11	1	1	6	4
	Radiation Therapy				Radiation Therapy				Radiation Therapy			
	Yes		No		Yes		No		Yes		No	
Non-use	0.89 (0.4-1.9)		1.0		0.42 (0.1-1.5)		1.0		2.72 (0.6-14.4)		1.0	
Ever	0.99 (0.2-3.1)		0.49 (0.2-0.9)		1.11 (0.2-5.9)		0.54 (0.2-1.2)		1.36 (0.1-45.5)		0.91 (0.2-4.0)	

[1] N_0=Disease-free survivors, N_1=Women with recurrences and/ or breast cancer mortality.

[1] Logistic regression models adjusted for age at diagnosis; age at menopause; tumor stage; Tamoxifen, radiation therapy use; hormone replacement therapy; smoking (past, current); Exercise (past, current) and dietary intake of vitamin C or E.

[2] Crude results presented due to limited sample size.